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(54) Title: ANTAGONISTIC SELECTIVE BINDING AGENTS OF OSTEOPROTEGERIN BINDING PROTEIN

(57) Abstract: Selective binding agents of osteoprotegerin binding protein (OPGbp) are provided by the invention. More particularly, the invention provides for antibodies and antigen binding domains which selectively bind to OPGbp and may be used to prevent or treat conditions relating to loss of bone mass. Nucleic acid molecules encoding said antibodies and antigen binding domains, and expression vectors and host cells for the production of same are also provided.

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## ANTAGONISTIC SELECTIVE BINDING AGENTS OF OSTEOPROTEGERIN BINDING PROTEIN

5 This application is a continuation-in-part of application Serial No. 09/511,139, pending, filed February 23, 2000, which is hereby incorporated by reference.

10 Field of the Invention

The invention relates to selective binding agents for osteoprotegerin binding protein (OPGbp). More particularly, the invention relates to antibodies and antigen binding domains which bind selectively to  
15 OPGbp and may be used to prevent or treat conditions relating to loss of bone mass. Nucleic acid molecules, vectors and host cells for the production of the selective binding agents of the invention are also provided.

20

Background of the Invention

Living bone tissue exhibits a dynamic equilibrium between deposition and resorption of bone.  
25 These processes are mediated primarily by two cell types: osteoblasts, which secrete molecules that comprise the organic matrix of bone; and osteoclasts, which promote dissolution of the bone matrix and solubilization of bone salts. In young individuals  
30 with growing bone, the rate of bone deposition exceeds the rate of bone resorption, while in older individuals the rate of resorption can exceed deposition. In the latter situation, the increased breakdown of bone leads to reduced bone mass and strength, increased risk of  
35 fractures, and slow or incomplete repair of broken bones.

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Osteoclasts are large phagocytic multinucleated cells which are formed from hematopoietic precursor cells in the bone marrow.

Although the growth and formation of mature functional osteoclasts is not well understood, it is thought that osteoclasts mature along the monocyte/macrophage cell lineage in response to exposure to various growth-promoting factors. Early development of bone marrow precursor cells to preosteoclasts are believed to be mediated by soluble factors such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), tumor necrosis factor- $\beta$  (TNF- $\beta$ ), interleukin-1 (IL-1), interleukin-4 (IL-4), interleukin-6 (IL-6), and leukemia inhibitory factor (LIF). In culture, preosteoclasts are formed in the presence of added macrophage colony stimulating factor (M-CSF). These factors act primarily in early steps of osteoclast development.

A polypeptide factor, osteoprotegerin binding protein (OPGbp), has been described which stimulates osteoclast formation and bone resorption and appears to act at a late stage of development. See PCT WO98/46751. OPGbp stimulates osteoclast formation from bone marrow precursor cells without the requirement for coculturing in the presence of a stromal cell line. Stimulation of bone resorption by OPGbp required interaction with its cognate receptor, osteoprotegerin differentiation and activation receptor (ODAR) and inhibition of the ODAR/OPGbp interaction by osteoprotegerin (OPG) also inhibited bone resorption. Consequently, the regulation of OPGbp binding to ODAR affects osteoclast formation and loss of bone.

It is an object of the invention to identify selective binding agents which regulate the interaction of OPGbp and ODAR, especially those agents which block the interaction of OPGbp and ODAR and/or inhibit at

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least one activity of OPGbp, such as bone resorption. It is a further object of the invention to identify those selective binding agents that may be used to prevent and treat loss of bone mass. It is a further  
5 object of the invention to identify an antibody, or an antigen binding domain, or a fragment or variant thereof, which regulates the interaction of OPGbp and ODAR and neutralizes at least one activity of OPGbp. The antibodies may be used to prevent and treat loss of  
10 bone mass.

#### Summary of the Invention

The invention provides for a selective  
15 binding agent of osteoprotegerin binding protein (OPGbp). In one embodiment, the selective binding agent of the invention partially or completely inhibits at least one activity of OPGbp; that is, the selective binding agent is an antagonist of OPGbp. In another  
20 embodiment, the selective binding agent binds to OPGbp in a manner that partially or completely inhibits the interaction of OPGbp with its cognate receptor, osteoclast differentiation and activation receptor, or ODAR, and thereby partially or completely inhibits  
25 OPGbp activity. Selective binding agents of the invention may be protein in nature and are referred to herein as proteinaceous selective binding agents.

The invention also provides for an antibody or antigen binding domain thereof, or a fragment,  
30 variant, or derivative thereof, which binds to an epitope on OPGbp and partially or completely inhibits at least one activity of OPGbp. That is, the antibody is an antagonist antibody. Preferably, OPGbp is mammalian OPGbp. More preferably, OPGbp is human OPGbp  
35 which may be in soluble or cell surface associated forms, or fragments, derivatives and variants thereof.



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When the selective binding agent is an antibody, such an antibody may be prepared by immunizing an animal with OPGbp such as murine or human OPGbp, preferably human OPGbp, or with an immunogenic fragment, derivative or variant thereof. In addition, an animal may be immunized with cells transfected with a vector containing a nucleic acid molecule encoding OPGbp such that OPGbp is expressed and associated with the surface of the transfected cells. Alternatively, selective binding agents which are antibodies may be obtained by screening a library comprising antibody or antigen binding domain sequences for binding to OPGbp. Such a library is conveniently prepared in bacteriophage as protein or peptide fusions to a bacteriophage coat protein which are expressed on the surface of assembled phage particles and the encoding DNA sequences contained within the phage particles (so-called "phage displayed library"). In one example, a phage displayed library contains DNA sequences encoding human antibodies, such as variable light and heavy chains.

Selective binding agents which are antibodies or antigen binding domains may be tetrameric glycoproteins similar to native antibodies, or they may be single chain antibodies; Fv, Fab, Fab' or F(ab)' fragments, bispecific antibodies, heteroantibodies, or other fragments, variants, or derivatives thereof, which are capable of binding OPGbp and partially or completely neutralize OPGbp activity. Antibodies or antigen binding domains may be produced in hybridoma cell lines (antibody-producing cells such as spleen cells fused to mouse myeloma cells, for example) or may be produced in heterologous cell lines transfected with nucleic acid molecules encoding said antibody or antigen binding domain.

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An antibody or antigen binding domain of the invention comprises:

(a) a Fab heavy chain amino acid sequence as shown in Figure 9 (SEQ ID NO: 51) or Figure 10 (SEQ ID NO: 53);

(b) a heavy chain amino acid sequence comprising conservative amino acid substitutions of the sequence in (a);

(c) a heavy chain amino acid sequence which is at least about 80% identical to the sequence in (a); or

(d) a fragment or derivative of (a), (b) or (c);

wherein the antibody or antigen binding domain binds selectively to OPGbp.

In another embodiment, an antibody or antigen binding domain of the invention recognizes an epitope on human OPGbp recognized by an antibody or antigen binding domain comprising a Fab heavy chain amino acid sequence as shown in Figure 9 (SEQ ID NO: 51) or Figure 10 (SEQ ID NO: 53) and a Fab light amino acid sequence as shown in Figure 5 (SEQ ID NO: 43) or Figure 6 (SEQ ID NO: 45). Also provided for is an anti-OPGbp antibody or antigen binding domain which recognizes a DE epitope on OPGbp.

In another embodiment, an antibody or antigen binding domain of the invention comprises a  $V_L$  and  $V_H$  chain:

wherein each  $V_L$  chain comprises CDR amino acid sequences designated CDR1( $V_L$ ), CDR2( $V_L$ ) and CDR3( $V_L$ ), separated by framework amino acid sequences, CDR1( $V_L$ ) being selected from the group consisting of:

RASQSIERYLN (SEQ ID NO: 01);

RASQSVGSYLA (SEQ ID NO: 02);

RASQSVSSSSLA (SEQ ID NO: 03); and

SGDALPKQY (SEQ ID NO: 04);

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CDR2(V<sub>1</sub>) being selected from the group consisting of:

GASSLQS (SEQ ID NO: 05);

DATNRAT (SEQ ID NO: 06);

GASSRAT (SEQ ID NO: 07); and

5 EDSERPS (SEQ ID NO: 08);

and CDR3(V<sub>1</sub>) being selected from the group consisting of:

QHTRA (SEQ ID NO: 09);

QHRRT (SEQ ID NO: 10);

10 QQYGA (SEQ ID NO: 11); and

QSTDSSGTYVV (SEQ ID NO: 12);

wherein CDR1(V<sub>1</sub>), CDR2(V<sub>1</sub>) and CDR3(V<sub>1</sub>) are selected independently of each other; and

wherein each V<sub>h</sub> chain comprises CDR amino acid sequences designated CDR1(V<sub>h</sub>), CDR2(V<sub>h</sub>) and CDR3(V<sub>h</sub>) separated by framework amino acid sequences, CDR1(V<sub>h</sub>) being selected from the group consisting of:

NYAIH (SEQ ID NO: 13);

NYPMH (SEQ ID NO: 14); and

20 DYAMH (SEQ ID NO: 15)

CDR2(V<sub>h</sub>) being selected from the group consisting of:

WINAGNGNTKFSQKFQG (SEQ ID NO: 16);

VISYDGNNKYADSVKG (SEQ ID NO: 17); and

GISWNSGRIGYADSVKG (SEQ ID NO: 18)

25 CDR3(V<sub>h</sub>) being selected from the group consisting of:

DSSNMVRGIIIIAYYFDY (SEQ ID NO: 19);

GGGGFDY (SEQ ID NO: 20); and

GGSTSARYSSGWYY (SEQ ID NO: 21)

wherein CDR1(V<sub>h</sub>), CDR2(V<sub>h</sub>) and CDR3(V<sub>h</sub>) are selected independently of each other.

In another embodiment, an antibody or antigen binding domain of the invention comprises a V<sub>1</sub> and a V<sub>h</sub> chain wherein:

the V<sub>1</sub> chain comprises CDR1 having the sequence RASQSIISRYLN (SEQ ID NO: 01), CDR2 having the

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sequence GASSLQS (SEQ ID NO: 05), and CDR3 having the sequence QHTRA (SEQ ID NO: 09); and

the  $V_L$  chain comprises CDR1 having the sequence NYAIH (SEQ ID NO: 13), CDR2 having the  
5 sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3 having the sequence DSSNMVRGIIIIAYYFDY (SEQ ID NO: 19);

wherein CDR1, CDR2 and CDR3 on each  $V_L$  and  $V_H$  chain are separated by framework amino acid sequences.

Antibodies and antigen binding domains of the  
10 invention are derived from germ line nucleic acid sequences present in genomic DNA which encode light and heavy chain amino acid sequences. Antibodies are encoded by nucleic acid sequences which are the products of germline sequence rearrangement and somatic  
15 mutation.

In one embodiment, an antibody or antigen binding domain of the invention comprises a  $V_L$  and a  $V_H$  chain wherein the  $V_L$  chain is comprises a rearranged or somatic variant of a  $V_{H1}$  germline genes such as in  
20 Figure 19 (SEQ ID NO: 66); and the  $V_H$  chain comprises a rearranged or somatic variant of a  $V_{H1}$  germline genes such as in Figure 16 (SEQ ID NO: 59); and the antibody binds selectively to an OPGbp polypeptide.

In another embodiment, the  $V_L$  chain comprises  
25 or a rearranged or somatic variant of a  $V_{K3}$  germline genes such as in Figure 20 (SEQ ID NO: 68); and the  $V_H$  chain comprises a rearranged or somatic variant of a  $V_{H1}$  germline gene such as in Figure 16 (SEQ ID NO: 59).

In another embodiment, the  $V_L$  chain comprises  
30 a rearranged or somatic variant of a  $V_{K3}$  germline gene such as in Figure 21 (SEQ ID NO: 70); and the  $V_H$  chain comprises a rearranged or somatic variant of a  $V_{H3}$  germline gene such as in Figure 17 (SEQ ID NO: 62).

In another embodiment, the  $V_L$  chain comprises  
35 a rearranged or somatic variant of a  $V_{L3}$  germline gene such as in Figure 22 (SEQ ID NO: 72); and the  $V_H$  chain

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comprises or a rearranged or somatic variant of a Vh3 germline gene such as in Figure 18 (SEQ ID NO: 64).

The selective binding agents of the invention (antibody or antigen binding domain) partially or completely inhibit at least one activity of OPGbp, such as binding of OPGbp to ODAR, formation or activation of osteoclasts, or OPGbp-mediated bone resorption and are used to prevent and/or treat bone diseases. In one embodiment, an OPGbp antagonist, such as an antibody or antigen binding domains, is administered to an animal which has experienced loss of bone mass, or is at risk for loss of bone mass, in order to prevent and/or treat loss of bone mass. An OPGbp antagonist may be used to prevent and/or treat osteoporosis, loss of bone mass due to metastasis of cancer to bone; loss of bone mass due to rheumatoid arthritis, hypercalcemia of malignancy and steroid-induced osteoporosis.

Also provided are compositions comprising the antibodies or antigen binding domains of the invention and a pharmaceutically acceptable carrier.

#### Description of the Figures

Figure 1 shows an ELISA of predominant Fab Patterns for reactivity to human OPGbp[143-317]. Titrations were performed using a maximum of 50  $\mu$ l of phage solution per well to given a typical range  $10^9$ - $10^{11}$  phage/well in the ELISA. Phage stocks for ELISA were prepared as described in Example 1. Values were from single point determinations. Patterns "AB" & "X" were superimposed on the same line.

Figure 2 shows inhibition of OPGbp binding to ODAR by Fabs "AT" and "Y". Fabs were purified as described in Example 4 and added to final well

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concentrations as shown in the figure. Details of the OPGbp/ODAR binding assay are set forth in Example 1. Values were averages of duplicate determinations.

5                Figure 3 shows bone marrow assays of Fabs "AT" "Y" & "P". The results of one endotoxin-free preparation (0.5 EU/ml or less) each of Fabs "AT", "Y" and "P" were shown. Fabs were purified as described in Example 4 and added to final well dilutions as shown in  
10 the figure (Fab stock solutions were 750 µg/ml to 1 mg/ml). The assay format includes a 1 hour pre-incubation of the anti-hu-OPGbp Fab with 10 ng/ml final cell well concentration of human OPGbp [143-317]. Values were averages of triplicate determinations.

15                Figure 4 shows Raw cell assays of Fabs "AT" "Y" & "P". The results of one endotoxin-free preparation (0.5 EU/ml or less) each of Fabs "AT", "Y" and "P" were shown. Fabs were purified as described in  
20 Example 4. Fabs were preincubated with human OPGbp [143-317] for 1 hour at room temperature before a 1/20 dilution to the final cell well concentration shown on the graph. The final cell well concentration of OPGbp was 20 ng/ml. The cell concentration was  $1 \times 10^5$  /ml.  
25 Values were from triplicate determinations with error bars designating 2 standard deviations (2 STD).

                    Figure 5 shows the nucleotide and amino acid sequence of Fab "AT" light chain.

30

                    Figure 6 shows the nucleotide and amino acid sequence of Fab "Y" light chain.

                    Figure 7 shows the nucleotide and amino acid  
35 sequence of Fab "P" light chain.



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Figure 8 shows the nucleotide and amino acid sequence of Fab "S" light chain.

Figure 9 shows the nucleotide and amino acid sequence of Fab "AT" heavy chain.

Figure 10 shows the nucleotide and amino acid sequence of Fab "Y" heavy chain.

Figure 11 shows the nucleotide and amino acid sequence of Fab "P" heavy chain.

Figure 12 shows the nucleotide and amino acid sequence of Fab "S" heavy chain.

Figure 13 shows a comparison of Fab amino acid sequences shown in Figures 5-12. The predicted amino acid sequences of heavy and light chain Fabs "AT", "Y", "P" and "S" were compared for identity and similarity. Heavy chains "AT" and "Y" differ at only one amino acid position. As library designed, all four Fabs have identical heavy chain CH1 regions comprising the carboxy half of the heavy chain which are included in the calculations of identity and similarity. Light chains "AT", "Y" and "P" share the same or similar V kappa families and therefore differ only at 1 to 2 amino acids in the carboxyl half of the chain, included in the calculations

Figure 14 shows a comparison of the predicted heavy and light chain complementarily determining regions (CDRs) of Fabs "AT", "Y", "P" and "S". For heavy chain comparisons, CDR1 includes amino acid residues 32-36 inclusive for all Fabs; CDR2 includes amino acid residues 51-67 inclusive for all Fabs; and CDR3 includes amino acid residues 100-116 inclusive for

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Fabs "AT" and "Y", 100-106 inclusive for Fab "P", and 100-113 inclusive for Fab "S". For light chain comparisons, CDR1 includes amino acid residues 29-39 inclusive for Fabs "AT" and "Y", 28-39 inclusive for Fab "P", and 27-35 inclusive for Fab "S"; CDR2 includes amino acid residues 55-61 inclusive for Fabs "AT", "Y", and "P", and 53-59 inclusive for Fab "S"; and CDR3 includes amino acid residues 94-98 inclusive for Fabs "AT", "Y" and "P" and 92-102 inclusive for Fab "S".

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Figure 15 shows a comparison of Fab classes. Fab class comparison was obtained from V-Base DNA PLOT analysis. The symbol (\*) indicates that the closest matching diversity (D) region, although related to known germ line sequences could not be determined. The symbol (\*\*) indicates that the germ line variable (V) region sequence of the closest match has been identified but not formally named to date, being of the rarer lambda family.

20

Figure 16 shows a comparison of predicted Fab "AT" and "Y" heavy chain amino acid sequences (residues 2-127 inclusive in Figures 9 and 10, respectively) with a germline sequence from the VH1 family. The germline sequence comprises the V region sequence 1-03, D region sequence 3-10, and J region sequence JH4 (SEQ. ID NO: 44). FR1, FR2 and FR3 designate the three framework regions, CDR1, CDR2, and CDR3 designate the three complementarily determining regions, and H1, H2 and H3 designate the corresponding junction sequences between framework regions and CDRs. Differences between "AT", "Y" and germline V, D, or J sequences are in boldface. The numbering of germline amino acid residues in Figures 16-22 is as described in Kabat et al. Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 4<sup>th</sup> ed. (1991).

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Figure 17 shows a comparison of predicted Fab "P" heavy chain amino acid sequences (residues 2-117 inclusive in Figure 11) with a germline sequence from the VH3 family. The sequence comprises the V region sequence 3-30 and the J region sequence JH4. The D region sequence is unknown.

Figure 18 shows a comparison of predicted Fab "S" heavy chain amino acid sequences (residues 2-124 inclusive in Figure 12) with a germline sequence from the VH3 family. The germline sequence comprises the V region sequence 3-09, D region sequence 6-19 and J regions sequence JH4.

Figure 19 shows a comparison of predicted Fab "AT" light chain amino acid sequence (residues 6-108 inclusive in Figure 5) with a germline sequence from the Vkappa1 family. The germline sequence comprises the V region sequence 012 and J region sequence JK1.

Figure 20 shows a comparison of predicted Fab "Y" light chain amino acid sequence (residues 6-108 inclusive in Figure 6) with a germline sequence from the Vkappa3 family. The germline sequence comprises the V region sequence L6 and the J region sequence JK2.

Figure 21 shows a comparison of predicted Fab "P" light chain amino acid sequence (residues 5-108 inclusive in Figure 7) with a germline sequence from the Vkappa3 family. The germline sequence comprises the V region sequence A27 and the J region sequence JK4.

Figure 22 shows a comparison of predicted Fab "S" light chain amino acid sequence (residues 5-112

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inclusive in Figure 8) with a germline sequence from the VL3 family. The germline sequence comprises the V region sequence 3m and the J region sequence JL2.

5                Figure 23 shows RAW cell assays of "AT" 405, "AT" 406 and "AT" 407 isolates. cDNA encoding Fab "AT" was fused to cDNA encoding CH1, CH2 and CH3 regions of human IgG1 as described in Example 7. Different leader sequences were used to produce the resulting isolates  
10 designated "AT" 405, "AT" 406 and "AT" 407. "AT" 405-407 were preincubated with OPGbp for 1 hour at room temperature before dilution to the final cell well concentration shown on the graph. The final cell well concentration of OPGbp was 40 ng/ml. Values were from  
15 triplicate determinations with error bars designating 2 standard deviations (2 STD).

              Figure 24 shows bone marrow assays of "AT" 405 and "AT" 407. The results of one endotoxin-free  
20 preparation (0.5 EU/ml or less) of "AT" 405 and "AT" 407 were shown. Samples were pre-incubated with human OPGbp [143-317] for 1 hour at room temperature before addition to the cells. The final cell well dilution for "AT" 405 and "AT" 407 from the sample stock is  
25 indicated on the x axis. The final cell well OPGbp concentration was 20 ng/ml.

              Figure 25 shows a bone marrow assay of "AT" 406. The results of one endotoxin-free preparation  
30 (0.5 EU/ml or less) of "AT" 406 is shown. Samples were pre-incubated with human OPGbp [143-317] for 1 hour at room temperature before addition to the cells. The final cell well concentration of the sample is indicated on the x axis. The final cell well  
35 concentration of OPGbp was 20 ng/ml.

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Figure 26 shows a bone marrow assay of "S" 435 and "Y" 429. Construction of "S" 435 and "Y" 429 are described in Example 7. The results of one endotoxin-free preparation (0.5 EU/ml or less) of each of "S" 435 and "Y" 429 are shown. Samples were pre-incubated with human OPGbp [143-317] for 1 hour at room temperature before addition to the cells. The final cell well concentration of the sample is indicated on the x axis. The final cell well concentration of OPGbp was 20 ng/ml.

Figure 27 shows a bone marrow assay of "Y" 442 and "P" 444. Construction and expression of "Y" 442 and "P" 444 is described in Example 7. The results of one endotoxin-free preparation (0.5 EU/ml or less) each of "Y" 442 and "P" 444 are shown. Samples were pre-incubated with human OPGbp [143-317] for 1 hour at room temperature before addition to the cells. The final cell well concentration of the sample is shown on the x axis. The final cell well concentration of OPGbp was 20 ng/ml.

Figure 28 shows the nucleic acid and amino acid sequence of FLAG-murine [153-316] OPGbp/DE.

Figure 29 is an alignment of human OPGbp[143-317], murine OPGbp[158-316], and FLAG-murine OPGbp [158-316]/DE amino acid sequences in the region of the DE loop. Underlined are the amino acid residues of human OPGbp introduced into mouse OPGbp to generate the FLAG-mouse OPGbp/DE molecule.

Figure 30 is an enzyme immunoassay examining the binding and reactivity of the AT antibody to plates coated with either human OPGbp[143-317], murine OPGbp[158-316], or FLAG-murine OPGbp [158-316]/DE.

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Detailed Description of the Invention

The present invention provides for agents which selectively bind OPG binding protein (OPGbp).

5 Preferably, the agents are OPGbp antagonists or inhibitors which inhibit partially or completely at least one activity of OPGbp, such as binding of OPGbp to its cognate receptor, ODAR, osteoclast formation and/or activation, or bone resorption. In one  
10 embodiment, the selective binding agent is an antibody which selectively binds OPGbp such that it partially or completely blocks the binding of OPGbp to its cognate receptor and partially or completely inhibits osteoclast formation and/or bone resorption.

15 The term "selective binding agent" refers to a molecule which preferentially binds OPGbp. A selective binding agent may include a protein, peptide, nucleic acid, carbohydrate, lipid, or small molecular weight compound. In a preferred embodiment, a  
20 selective binding agent is an antibody, such as polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, CDR-grafted antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as  
25 fragments, regions or derivatives thereof, provided by known techniques, including, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques. The anti-OPGbp selective binding agents of the present invention are capable of binding portions  
30 of OPGbp that inhibit the binding of OPGbp to ODAR receptors.

The antibodies and antigen binding domains of the invention bind selectively to OPGbp, that is they bind preferentially to OPGbp with a greater binding  
35 affinity than to other antigens. The antibodies may bind selectively to human OPGbp, but also bind



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detectably to non-human OPGbp, such as murine OPGbp. Alternatively, the antibodies may bind selectively to non-human OPG, but also bind detectably to human OPG. Alternatively, the antibodies may bind exclusively to human OPGbp, with no detectable binding to non-human OPGbp.

The term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies wherein each monoclonal antibody will typically recognize a single epitope on the antigen. The term "monoclonal" is not limited to any particular method for making the antibody. For example, monoclonal antibodies of the invention may be made by the hybridoma method as described in Kohler et al. Nature 256, 495 (1975) or may be isolated from phage libraries using the techniques as described herein, for example.

The term "antigen binding domain" or "antigen binding region" refers to that portion of the selective binding agent (such as an antibody molecule) which contains the amino acid residues that interact with an antigen and confer on the binding agent its specificity and affinity for the antigen. Preferably, the antigen binding region will be of human origin. In other embodiments, the antigen binding region can be derived from other animal species, in particular rodents such as rabbit, rat or hamster.

The term "epitope" refers to that portion of any molecule capable of being recognized by and bound by a selective binding agent (such as an antibody) at one or more of the binding agent's antigen binding regions. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics. By "inhibiting and/or neutralizing

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epitope" is intended an epitope, which, when bound by a selective binding agent, results in loss of biological activity of the molecule or organism containing the epitope, *in vivo*, *in vitro*, or *in situ*, more preferably *in vivo*, including binding of OPGbp to its receptor.

The term "light chain" when used in reference to an antibody refers to two distinct types, called kappa ( $\kappa$ ) or lambda ( $\lambda$ ) based on the amino acid sequence of the constant domains.

The term "heavy chain" when used in reference to an antibody refers to five distinct types, called alpha, delta, epsilon, gamma and mu, based on the amino acid sequence of the heavy chain constant domain. These distinct types of heavy chains give rise to five classes of antibodies, IgA, IgD, IgE, IgG and IgM, respectively, including four subclasses of IgG, namely IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, and IgG<sub>4</sub>.

The term "variable region" or "variable domain" refers to a portion of the light and heavy chains, typically about the amino-terminal 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, which differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarily determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). The CDRs of the light and heavy chains are responsible for the interaction of the antibody with antigen.

The term "constant region" or "constant domain" refers to a carboxy terminal portion of the light and heavy chain which is not directly involved in binding of the antibody to antigen but exhibits various

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effector function, such as interaction with the Fc receptor.

The term "OPGbp" or "OPGbp polypeptide" refers to a polypeptide comprising the amino acid sequence as shown in Figure 4 of PCT publication WO/46757, the disclosure of which is incorporated by reference, and related polypeptides. Related polypeptides include allelic variants; splice variants; fragments; derivatives; substitution, deletion, and insertion variants; fusion polypeptides; and interspecies homologs. Also encompassed are soluble forms of OPGbp, such as residues 69-317 inclusive of human OPGbp (as numbered in WO 98/46757), or a subset thereof which is sufficient to generate an immunological response. In one embodiment, soluble human OPGbp includes residues 140-317 inclusive, 143-317 inclusive, or immunogenic fragments thereof. OPGbp may be a mature polypeptide, as defined herein, and may or may not have an amino terminal methionine residue, depending upon the method by which it is prepared.

The term "fragment" when used in relation to OPGbp or to a proteinaceous selective binding agent of OPGbp refers to a peptide or polypeptide that comprises less than the full length amino acid sequence. Such a fragment may arise, for example, from a truncation at the amino terminus, a truncation at the carboxy terminus, and/or an internal deletion of a residue(s) from the amino acid sequence. Fragments may result from alternative RNA splicing or from *in vivo* protease activity.

The term "variant" when used in relation to OPGbp or to a proteinaceous selective binding agent of OPGbp refers to a peptide or polypeptide comprising one or more amino acid sequence substitutions, deletions, and/or additions as compared to a native or unmodified sequence. For example, an OPGbp variant may result

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from one or more changes to an amino acid sequence of native OPGbp. Also by way of example, a variant of a selective binding agent of OPGbp may result from one or more changes to an amino acid sequence of a native or previously unmodified selective binding agent.

5 Variants may be naturally occurring, such as allelic or splice variants, or may be artificially constructed. Polypeptide variants may be prepared from the corresponding nucleic acid molecules encoding said variants.

10 The term "derivative" when used in relation to OPGbp or to a proteinaceous selective binding agent of OPGbp refers to a polypeptide or peptide, or a variant, fragment or derivative thereof, which has been chemically modified. Examples include covalent attachment of one or more polymers, such as water soluble polymers, N-linked, or O-linked carbohydrates, sugars, phosphates, and/or other such molecules. The derivatives are modified in a manner that is different from naturally occurring or starting peptide or polypeptides, either in the type or location of the molecules attached. Derivatives further include deletion of one or more chemical groups which are naturally present on the peptide or polypeptide.

25 The term "fusion" when used in relation to OPGbp or to a proteinaceous selective binding agent of OPGbp refers to the joining of a peptide or polypeptide, or fragment, variant and/or derivative thereof, with a heterologous peptide or polypeptide.

30 The term "biologically active" when used in relation to OPGbp or to a proteinaceous selective binding agent refers to a peptide or a polypeptide having at least one activity characteristic of OPGbp or a selective binding agent. A selective binding agent of OPGbp may have agonist, antagonist, or neutralizing

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or blocking activity with respect to at least one biological activity of OPGbp.

5 The term "naturally occurring" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to those which are found in nature and not manipulated by a human being.

10 The term "isolated" when used in relation to OPGbp or to a proteinaceous selective binding agent of OPGbp refers to a peptide or polypeptide that is free from at least one contaminating polypeptide that is found in its natural environment, and preferably substantially free from any other contaminating mammalian polypeptides which would interfere with its  
15 therapeutic or diagnostic use.

The term "mature" when used in relation to OPGbp or to a proteinaceous selective binding agent of OPGbp refers to a peptide or polypeptide lacking a leader sequence. The term may also include other  
20 modifications of a peptide or polypeptide such as proteolytic processing of the amino terminus (with or without a leader sequence) and/or the carboxy terminus, cleavage of a smaller polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and  
25 the like.

The terms "effective amount" and "therapeutically effective amount" when used in relation to a selective binding agent of OPGbp refers to an amount of a selective binding agent that is  
30 useful or necessary to support an observable change in the level of one or more biological activities of OPGbp. Said change may be either an increase or decrease in the level of OPGbp activity.

The term "conservative amino acid substitution"  
35 refers to a substitution of a native amino acid residue with a non-native residue such that there is little or

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no effect on the polarity or charge of the amino acid residue at that position. For example, a conservative substitution results from the replacement of a non-polar residue in a polypeptide with any other non-polar residue. Furthermore, any native residue in a polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (Cunningham et al. Science 244, 1081-1085 (1989)). Exemplary rules for conservative amino acid substitutions are set forth in Table I.

Table I  
Conservative Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Ala



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Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Conservative modifications to the amino acid sequence (and the corresponding modifications to the encoding nucleotides) are able to produce OPGbp polypeptides (and proteinaceous selective binding agents thereof) having functional and chemical characteristics similar to those of naturally occurring OPGbp or selective binding agents. In contrast, substantial modifications in the functional and/or chemical characteristics of OPGbp (and proteinaceous selective binding agents thereof) may be accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues may be divided into groups based on common side chain properties:

- 1) Hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- 2) Neutral hydrophilic: Cys, Ser, Thr;

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- 3) Acidic: Asp, Glu;
- 4) Basic: Asn, Gln, His, Lys, Arg;
- 5) Residues that influence chain orientation:  
Gly, Pro; and
- 5 6) Aromatic: Trp, Tyr, Phe.

Non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class.

10 The "identity or similarity" of two or more nucleic acid molecules and/or polypeptides provides a measure of the relatedness of two or more distinct sequences. The term "identity" refers to amino acids which are identical at corresponding positions in two distinct amino acid sequences. The term "similarity"

15 refers to amino acids which are either identical or are conservative substitutions as defined above at corresponding positions in two distinct amino acid sequences.

The extent of identity or similarity can be

20 readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York,

25 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds.,

30 M. Stockton Press, New York, 1991; and Carillo et al., *SIAM J. Applied Math.*, 48, 1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine

35 identity and similarity are codified in publicly available computer programs. Exemplary computer

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program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux et al., Nucleic Acids Research 12, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol., 215, 403-410 (1990)). The BLAST X program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB NLM NIH Bethesda, MD). The well known Smith Waterman algorithm may also be used to determine identity.

15 OPGbp polypeptides

OPGbp polypeptides, and fragments, variants and derivatives thereof, are used as target molecules for screening and identifying the selective binding agents of the invention. When it is desired to prepare antibodies as selective binding agents, OPGbp polypeptides are preferably immunogenic, that is they elicit an immune response when administered to an animal. Alternatively, when antibodies are prepared by in vitro techniques, OPGbp polypeptides used as target molecules are capable of detectably binding an antibody or antigen binding domain.

OPG polypeptides are prepared by biological or chemical methods. Biological methods such as expression of DNA sequences encoding recombinant OPGbp are known in the art (see for example Sambrook et al. supra). Chemical synthesis methods such as those set forth by Merrifield et al., J. Am. Chem. Soc., 85:2149 (1963), Houghten et al., Proc Natl Acad. Sci. USA, 82:5132 (1985), and Stewart and Young, Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL (1984) may also be used to prepare OPGbp polypeptides

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of the invention. Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized OPGbp polypeptides, or fragments or variants thereof, may be oxidized using methods set forth in these references to form disulfide bridges. OPGbp polypeptides of the invention prepared by chemical synthesis will have at least one biological activity comparable to the corresponding OPGbp polypeptides produced recombinantly or purified from natural sources.

OPGbp polypeptides may be obtained by isolation from biological samples such as source tissues and/or fluids in which the OPGbp polypeptides are naturally found. Sources for OPGbp polypeptides may be human or non-human in origin. Isolation of naturally-occurring OPGbp polypeptides can be accomplished using methods known in the art, such as separation by electrophoresis followed by electroelution, various types of chromatography (affinity, immunoaffinity, molecular sieve, and/or ion exchange), and/or high pressure liquid chromatography. The presence of the OPGbp polypeptide during purification may be monitored using, for example, an antibody prepared against recombinantly produced OPGbp polypeptide or peptide fragments thereof.

Polypeptides of the invention include isolated OPGbp polypeptides and polypeptides related thereto including fragments, variants, fusion polypeptides, and derivatives as defined hereinabove. OPGbp fragments of the invention may result from truncations at the amino terminus (with or without a leader sequence), truncations at the carboxy terminus, and/or deletions internal to the polypeptide. Such OPGbp polypeptides fragments may optionally comprise an amino terminal methionine residue. The polypeptides of

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the invention will be immunogenic in that they will be capable of eliciting an antibody response.

OPGbp polypeptide variants of the invention include one or more amino acid substitutions, additions and/or deletions as compared to the native OPGbp amino acid sequence. Amino acid substitutions may be conservative, as defined above, or non-conservative or any combination thereof. The variants may have additions of amino acid residues either at the carboxy terminus or at the amino terminus (where the amino terminus may or may not comprise a leader sequence).

Embodiments of the invention include OPGbp glycosylation variants and cysteine variants. OPGbp glycosylation variants include variants wherein the number and/or type of glycosylation sites has been altered compared to native OPGbp polypeptide. In one embodiment, OPGbp glycosylation variants comprise a greater or a lesser number of N-linked glycosylation sites compared to native OPGbp. Also provided for are OPGbp glycosylation variants comprising a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. OPGbp cysteine variants comprise a greater number or alternatively a lesser number of cysteine residues compared to native OPGbp. In one embodiment, one or more cysteine residues are deleted or substituted with another amino acid (e.g., serine). Cysteine variants of OPGbp can improve the recovery of biologically active OPGbp by aiding the refolding of OPGbp into a biologically active conformation after isolation from a denatured state.

Preparing OPGbp polypeptide variants is within the level of skill in the art. In one approach, one may introduce one or more amino acid substitutions, deletions and/or additions in native

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OPGbp wherein the OPGbp variant retains the native structure of OPGbp and/or at least one of the biological activities. One approach is to compare sequences of OPG polypeptides from a variety of  
5 different species in order to identify regions of relatively low and high identity and/or similarity. It is appreciated that those regions of an OPGbp polypeptide having relatively low identity and/or similarity, are less likely to be essential for  
10 structure and activity and therefore may be more tolerant of amino acid alterations, especially those which are non-conservative. It is also appreciated that even in relatively conserved regions, one could introduce conservative amino acid substitutions while  
15 retaining activity.

In another approach, structure-function relationships can be used to identify residues in similar polypeptides that are important for activity or structure. For example, one may compare conserved  
20 amino acid residue among OPGbp and other members of the tumor necrosis factor family for which structure-function analyses are available and, based on such a comparison, predict which amino acid residues in OPGbp are important for activity or structure. One skilled  
25 in the art may choose chemically similar amino acid substitutions for such predicted important amino acid residues of OPGbp.

In yet another approach, an analysis of a secondary or tertiary structure of OPGbp (either  
30 determined by x-ray diffraction of OPGbp crystals or by structure prediction methods) can be undertaken to determine the location of specific amino acid residues in relation to actual or predicted structures within an OPGbp polypeptide. Using this information, one can  
35 introduce amino acid changes in a manner that seeks to



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retain as much as possible the secondary and/or tertiary structure of an OPGbp polypeptide.

In yet another approach, the effects of altering amino acids at specific positions may be tested experimentally by introducing amino acid substitutions and testing the altered OPGbp polypeptides for biological activity using assays described herein. Techniques such as alanine scanning mutagenesis (Cunningham et al., supra) are particularly suited for this approach. Many altered sequence may be conveniently tested by introducing many substitutions at various amino acid positions in OPGbp and screening the population of altered polypeptides as part of a phage display library. Using this approach, those regions of an OPGbp polypeptide that are essential for activity may be readily determined.

The above methods are useful for generating OPGbp variants which retain the native structure. Thus, antibodies raised against each variants are likely to recognize a native structural determinant, or epitope, of OPGbp and are also likely to bind to native OPGbp. However, in some cases it may be desirable to produce OPGbp variants which do not retain native OPGbp structure or are partially or completely unfilled. Antibodies raised against such proteins will recognize buried epitopes on OPGbp.

The invention also provides for OPGbp fusion polypeptides which comprise OPGbp polypeptides, and fragments, variants, and derivatives thereof, fused to a heterologous peptide or protein. Heterologous peptides and proteins include, but are not limited to: an epitope to allow for detection and/or isolation of a OPGbp fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain, or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a

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transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a protein or peptide which promotes oligomerization, such as leucine zipper domain; and a protein or peptide which increases stability, such as an immunoglobulin constant region. A OPGbp polypeptide may be fused to itself or to a fragment, variant, or derivative thereof. Fusions may be made either at the amino terminus or at the carboxy terminus of a OPGbp polypeptide, and may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for separation of the fused moieties.

15 In a further embodiment of the invention, a OPGbp polypeptide, fragment, variant and/or derivative is fused to an Fc region of human IgG. In one example, a human IgG hinge, CH2 and CH3 region may be fused at either the N-terminus or C-terminus of the OPGbp polypeptides using methods known to the skilled artisan. In another example, a portion of a hinge regions and CH2 and CH3 regions may be fused. The OPGbp Fc-fusion polypeptide so produced may be purified by use of a Protein A affinity column. In addition, peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life in vivo than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, reduce aggregation, etc.

OPGbp polypeptide derivatives are included in the scope of the present invention. Such derivatives are chemically modified OPGbp polypeptide compositions in which OPGbp polypeptide is linked to a polymer. The

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polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. The polymer may be of any molecular weight, and may be branched or unbranched. Included within the scope of OPGbp polypeptide polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

10           The water soluble polymer or mixture thereof may be for example, polyethylene glycol (PEG), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran, of, for example about 6 kD), cellulose, or other carbohydrate based polymers, poly-  
15 (N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol.

          A preferred water soluble polymer is  
20 polyethylene glycol. As used herein, polyethylene glycol is meant to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono- ( $C_1$ - $C_{10}$ ) alkoxy-, or aryloxy-polyethylene glycol. Also encompassed by the invention are  
25 bifunctional PEG crosslinking molecules which may be used to prepare covalently attached OPGbp multimers.

          Methods for preparing chemically derivatized OPGbp polypeptides are known in the art. By way of example, derivatization of OPGbp polypeptides with PEG  
30 may be carried out using procedures described in Francis *et al.*, *Focus on Growth Factors*, 3, 4-10 (1992); EP 0 154 316; EP 0 401 384, and U.S. Patent No. 4,179,337. In a preferred embodiment, an OPGbp polypeptide derivative will have a single PEG moiety at  
35 the amino terminus. See U.S. Patent No. 5,234,784, herein incorporated by reference.

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OPGbp polypeptide derivatives disclosed herein may exhibit an enhancement or reduction of at least one biological activity of OPGbp compared to unmodified polypeptide, or may exhibit increased or  
5 decreased half-life or stability.

OPGbp selective binding agents

OPGbp polypeptides, and fragments, variants  
10 and derivatives thereof, may be used to identify selective binding agents of OPGbp. As defined above, a selective binding agent of OPGbp encompasses both proteinaceous and non-proteinaceous binding agents and, in one preferred embodiment of the invention, the  
15 selective binding agent is proteinaceous. In yet another preferred embodiment, the selective binding agent is an antibody or fragment thereof which binds OPGbp, preferably human OPGbp. The antibodies of the invention may be agonist antibodies, which enhance the  
20 level of at least one biological activity of OPGbp; or antagonist antibodies, which decrease the level of at least one biological activity of OPGbp. Antagonist antibodies of OPGbp may also be referred to as inhibitory or neutralizing antibodies of OPGbp.  
25 Although such antibodies are preferred embodiments of the invention, it is understood that other proteinaceous selective binding agents which are agonists or antagonists of OPGbp activity are also encompassed by the invention.

30 As described in the examples below, anti-OPGbp antibodies and antigen binding domains which inhibit at least one activity of OPGbp have been identified. Embodiments of the invention include antibodies comprising a heavy chain Fab sequence as  
35 shown in any of Figures 9, 10, 11 or 12 and further comprising a kappa or lambda light chain sequence.

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Light chain Fab sequences may be as shown in Figures 5, 6, 7 or 8. For example, "AT" antibody has light and heavy chain sequences in Figures 5 and 9, respectively; "Y" antibody has light and heavy chains sequences of Figures 6 and 10, respectively; "S" antibody has light and heavy chain sequences of Figures 7 and 11, respectively; and "P" antibody has light and heavy chain sequences of Figures 8 and 12, respectively. The antibodies of the invention further comprise a human Fc region from any isotype, either IgG, IgM, IgA, IgE, or IgD. Preferably, the Fc region is from human IgG, such as IgG1, IgG2, IgG3, or IgG4.

The invention also provides for antibodies or antigen binding domains which comprise fragments, variants, or derivatives of the Fab sequences disclosed herein. Fragments include variable domains of either the light or heavy chain Fab sequences which are typically joined to light or heavy constant domains. Variants include antibodies comprising light chain Fab sequences which are at least about 80%, 85%, 90%, 95%, 98% or 99% identical or similar to the Fab sequences, or the corresponding variable domains, in any one of Figures 5-8, or antibodies comprising heavy chain Fab sequences, or the corresponding variable domains, which are at least about 80%, 85%, 90%, 95%, 98% or 99% identical or similar to the Fab sequences in any one of Figures 9-12. The antibodies may be typically associated with constant regions of the heavy and light chains to form full-length antibodies.

Antibodies and antigen binding domains, and fragments, variants and derivatives thereof, of the invention will retain the ability to bind selectively to an OPGbp polypeptide, preferably to a human OPGbp polypeptide. In one embodiment, an antibody will bind an OPGbp polypeptide with a dissociation constant (KD) of about 1 nM or less, or alternatively 0.1 nM or less,



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or alternatively 10 pM or less or alternatively less than 10 pM. In Example 8, it was observed that "AT" antibody binds to OPGbp with a KD of about 0.33 to 0.43 nM.

5                   Antibodies of the invention include polyclonal monospecific polyclonal, monoclonal, recombinant, chimeric, humanized, fully human, single chain and/or bispecific antibodies. Antibody fragments include those portions of an anti-OPGbp antibody which  
10 bind to an epitope on an OPGbp polypeptide. Examples of such fragments include Fab F(ab'), F(ab)'', Fv, and sFv fragments. The antibodies may be generated by enzymatic cleavage of full-length antibodies or by recombinant DNA techniques, such as expression of  
15 recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

                  Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. An antigen is a  
20 molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen can have one or more epitope. The specific reaction  
25 referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens.

                  Polyclonal antibodies directed toward an  
30 OPGbp polypeptide generally are raised in animals (e.g., rabbits or mice) by multiple subcutaneous or intraperitoneal injections of OPGbp and an adjuvant. In accordance with the invention, it may be useful to conjugate an OPGbp polypeptide, or a variant, fragment,  
35 or derivative thereof to a carrier protein that is immunogenic in the species to be immunized, such as



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keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-OPGbp antibody titer.

Monoclonal antibodies (mAbs) contain a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. A hybridoma producing a monoclonal antibody of the present invention may be cultivated *in vitro*, *in situ*, or *in vivo*. Production of high titers *in vivo* or *in situ* is a preferred method of production.

Monoclonal antibodies directed toward OPGbp are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include hybridoma methods of Kohler et al., *Nature* 256, 495-497 (1975), and the human B-cell hybridoma method, Kozbor, J. *Immunol.* 133, 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988); the contents of which references are incorporated entirely herein by reference.

Preferred anti-OPGbp selective binding agents include monoclonal antibodies which will inhibit partially or completely the binding of human OPGbp to its cognate receptor, ODAR, or an antibody having substantially the same specific binding characteristics, as well as fragments and regions

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thereof. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.*, 92:589-601 (1983). These references are incorporated herein by reference.

Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with OPGbp polypeptides.

Chimeric antibodies are molecules in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine monoclonal antibodies have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric monoclonal antibodies are used.

Chimeric antibodies and methods for their production are known in the art. Cabilly et al., *Proc. Natl. Acad. Sci. USA*, 81:3273-3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984); Boulianne et al., *Nature*, 312:643-646 (1984); Neuberger et al., *Nature*, 314:268-270 (1985); Liu et al., *Proc. Natl. Acad. Sci. USA*, 84:3439-3443 (1987); and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988). These references are incorporated herein by reference.

For example, chimeric monoclonal antibodies of the invention may be used as a therapeutic. In such a chimeric antibody, a portion of the heavy and/or light

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chain is identical with or homologous to corresponding sequence in antibodies derived from a particular species or belonging to one particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci.*, 81, 6851-6855 (1985)).

As used herein, the term "chimeric antibody" includes monovalent, divalent or polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer (HL) formed by a chimeric H chain associated through disulfide bridges with a chimeric L chain. A divalent chimeric antibody is tetramer ( $H_2L_2$ ) formed by two HL dimers associated through at least one disulfide bridge. A polyvalent chimeric antibody can also be produced, for example, by employing a  $C_H$  region that aggregates (e.g., from an IgM H chain, or  $\mu$  chain).

Murine and chimeric antibodies, fragments and regions of the present invention may comprise individual heavy (H) and/or light (L) immunoglobulin chains. A chimeric H chain comprises an antigen binding region derived from the H chain of a non-human antibody specific for OPGbp, which is linked to at least a portion of a human H chain C region ( $C_H$ ), such as  $CH_1$  or  $CH_2$ .

A chimeric L chain according to the present invention comprises an antigen binding region derived from the L chain of a non-human antibody specific for OPGbp, linked to at least a portion of a human L chain C region ( $C_L$ ).

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Selective binding agents, such as antibodies, fragments, or derivatives, having chimeric H chains and L chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to known method steps, e.g., according to Ausubel et al., eds. *Current Protocols in Molecular Biology*, Wiley Interscience, N.Y. (1993), and Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988). The contents of these references are incorporated entirely herein by reference. With this approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

As an example, the antigen binding region of the selective binding agent (such as a chimeric antibody) of the present invention is preferably derived from a non-human antibody specific for human OPGbp. Preferred sources for the DNA encoding such a non-human antibody include cell lines which produce antibodies, such as hybrid cell lines commonly known as hybridomas.

The invention also provides for fragments, variants and derivatives, and fusions of anti-OPGbp antibodies, wherein the terms "fragments", "variants", "derivatives" and "fusions" are defined herein. The invention encompasses fragments, variants, derivatives, and fusions of anti-OPGbp antibodies which are functionally similar to the unmodified anti-OPGbp

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antibody, that is, they retain at least one of the activities of the unmodified antibody. In addition to the modifications set forth above, also included is the addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins. The fragments, variants, derivatives and fusions of anti-OPGbp antibodies can be produced from any of the hosts of this invention.

Suitable fragments include, for example, Fab, Fab', F(ab')<sub>2</sub>, Fv and scFv. These fragments lack the Fc fragment of an intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody. See Wahl et al., *J. Nucl. Med.*, 24:316-325 (1983). These fragments are produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). The identification of these antigen binding regions and/or epitopes recognized by monoclonal antibodies of the present invention provides the information necessary to generate additional monoclonal antibodies with similar binding characteristics and therapeutic or diagnostic utility that parallel the embodiments of this application.

The invention provides for anti-OPGbp antibodies, or antigen binding domains, which recognize and bind to inhibiting and/or neutralizing epitopes on OPGbp. As a result of this binding, an anti-OPGbp antibody may partially or completely inhibit binding of OPGbp to its receptor, or may partially or completely inhibit osteoclast formation, bone resorption and/or bone loss. More particularly, the invention provides for anti-OPGbp antibodies which recognize and bind to an epitope comprising a portion of the amino acid sequence of a DE region of OPGbp (a "DE epitope"). A



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DE region of OPGbp spans approximately the D and E beta sheet regions and intervening loop sequence (a "DE loop"). The DE region in human OPGbp comprises from about amino acid residue 212 to about amino acid residue 250 inclusive (see Figure 29). However, the amino acid sequence and endpoints of the DE region of human OPGbp are merely exemplary, and it is understood that DE regions may have sequences and endpoints which vary from those in human OPGbp. The invention encompasses antibodies which bind to such variable DE regions.

While it is contemplated that an anti-OPGbp antibody, or an antigen binding domain, may bind at any location within a DE region, a preferred embodiment is an anti-OPGbp antibody which binds to at least part of a DE loop. The DE loop in human OPGbp spans approximately five amino acids and is located at about residues 230-234 inclusive. The DE loop in human OPGbp has the sequence DLATE. However, the amino acid sequence and endpoints of the DE loop of human OPGbp are merely exemplary and it is understood that DE loops could have sequences and endpoints which vary from those in human OPGbp. The invention encompass antibodies which bind to such variable DE loops.

As shown in Example 10, introduction of the sequence DLATE into the corresponding DE loop of murine OPGbp resulted in binding of "AT" antibody whereas the antibody had no detectable affinity for murine OPGbp with the native DE loop sequence up to an antibody concentration of about 2  $\mu$ g/ml. In another embodiment, an anti-OPGbp antibody binds to the amino acid sequence DLATE in human OPGbp, or to a portion of said sequence. In another embodiment, an anti-OPGbp antibody, or antigen binding domain, binds to murine OPGbp comprising the amino acid substitutions S229D, V230L,



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P231A and D233E, but does not bind to murine OPGbp lacking said substitutions under similar conditions.

While the antibodies of the invention are characterized in part by the amino acid sequences on OPGbp to which they bind, it is understood and appreciated by one skilled in the art that a DE epitope on OPGbp recognized by an antibody typically comprises a three dimensional structure which may involve amino acids outside the DE region. In a linear representation of an OPGbp sequence, amino acids comprising the DE epitope may be distant from the DE region, but in a three dimensional structure of OPGbp, amino acids of the DE epitope will likely be in proximity to the DE region. Thus, it is understood that binding of an anti-OPGbp antibody to a DE epitope may involve amino acids other than those in the DE region. Nonetheless, it has been shown that amino acid residues in the DE loop, especially some or all of the residues in the sequence DLATE, are involved in antibody binding to OPGbp and inhibition of OPGbp activity.

Variants of selective binding agents are also provided. In one embodiment, variants of antibodies and antigen binding domains comprise changes in light and/or heavy chain amino acid sequences that are naturally occurring or are introduced by in vitro engineering of native sequences using recombinant DNA techniques. Naturally occurring variants include "somatic" variants which are generated in vivo in the corresponding germ line nucleotide sequences during the generation of an antibody response to a foreign antigen. Variants encoded by somatic mutations in germline variable light and heavy chain sequences which generate the exemplary Fabs of the present invention in sequences are shown in Figures 16 and 19 for Fab "AT";

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Figures 16 and 20 for Fab "Y", Figures 17 and 21 for Fab "P" and Figures 18 and 22 for Fab "S".

5 Variants of anti-OPGbp antibodies and antigen binding domains are also prepared by mutagenesis techniques known in the art. In one example, amino acid changes may be introduced at random throughout an antibody coding region and the resulting variants may be screened for a desired activity, such as binding affinity for OPGbp. Alternatively, amino acid changes  
10 may be introduced in selected regions of an OPGbp antibody, such as in the light and/or heavy chain CDRs, and framework regions, and the resulting antibodies may be screened for binding to OPGbp or some other activity. Amino acid changes encompass one or more  
15 amino acid substitutions in a CDR, ranging from a single amino acid difference to the introduction of all possible permutations of amino acids within a given CDR, such as CDR3. In another method, the contribution of each residue within a CDR to OPGbp binding may be  
20 assessed by substituting at least one residue within the CDR with alanine (Lewis et al., Mol. Immunol. 32, 1065-1072 (1995)). Residues which are not optimal for binding to OPGbp may then be changed in order to determine a more optimum sequence. Also encompassed  
25 are variants generated by insertion of amino acids to increase the size of a CDR, such as CDR3. For example, most light chain CDR3 sequences are nine amino acids in length. Light chain CDR3 sequences in an antibody which are shorter than nine residues may be optimized  
30 for binding to OPGbp by insertion of appropriate amino acids to increase the length of the CDR.

In one embodiment, antibody or antigen binding domain variants comprise one or more amino acid changes in one or more of the heavy or light chain  
35 CDR1, CDR2 or CDR3 and optionally one or more of the heavy or light chain framework regions FR1, FR2 or FR3.

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Amino acid changes comprise substitutions, deletions and/or insertions of amino acid residues. Exemplary variants include an "AT" heavy chain variable region variant with one or more amino acid changes in the sequences NYAIH (SEQ ID NO: 13); WINAGNGNTIKFSQKFQF (SEQ ID NO: 16); or DSSNMVRGIIIAYYFDY (SEQ ID NO: 19), or an "AT" light chain variable region variant with one or more amino acid changes in the sequences RASQISIRYLN (SEQ ID NO: 01); GASSLQS (SEQ ID NO: 05); or QHTRA (SEQ ID NO: 09). The aforementioned "AT" heavy and light chain variable region variants may further comprise one or more amino acid changes in the framework regions. In one example, one or more amino acid changes may be introduced to substitute a somatically mutated framework residue with the germline residue at that position. When the aforementioned amino acid changes are substitutions, the changes may be conservative or non-conservative substitutions.

Examples 11 and 12 provide variants in light and heavy chain CDR3 region of AT antibody. In one embodiment, the invention provides for variants in either SEQ ID NO:19 (heavy chain CDR3) or SEQ ID NO:9 (light chain CDR3) such that the resulting antibodies or antigen binding domains bind selectively to an OPG binding protein. In one embodiment, the OPGbp is human OPGbp.

The invention provides for anti-OPG bp antibodies comprising variable light and variable heavy chains and further comprising a heavy chain CDR3 region having the sequence selected from the group consisting of:

XSSNMVRGIIIAYYFDY (SEQ ID NO: 80);  
DXSNMVRGIIIAYYFDY (SEQ ID NO: 81);  
DSXNMVRGIIIAYYFDY (SEQ ID NO: 82);  
DSSXMVRGIIIAYYFDY (SEQ ID NO: 83);

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5 DSSNXVRGIIIIAYYFDY (SEQ ID NO: 84);  
 DSSNMXRGIIIIAYYFDY (SEQ ID NO: 85);  
 DSSNMVXGIIIIAYYFDY (SEQ ID NO: 86);  
 DSSNMVRXIIIIAYYFDY (SEQ ID NO: 87);  
 DSSNMVRGXIIIIAYYFDY (SEQ ID NO: 88);  
 DSSNMVRGIXIIIIAYYFDY (SEQ ID NO: 89);  
 DSSNMVRGIIIXAYYFDY (SEQ ID NO: 90);  
 DSSNMVRGIIIXYYFDY (SEQ ID NO: 91);  
 DSSNMVRGIIIIAXYFDY (SEQ ID NO: 92);  
 10 DSSNMVRGIIIIAYXFDY (SEQ ID NO: 93);  
 DSSNMVRGIIIIAYYXDY (SEQ ID NO: 94);  
 DSSNMVRGIIIIAYYFXY (SEQ ID NO: 95); and  
 DSSNMVRGIIIIAYYFDX (SEQ ID NO: 96);

15 wherein X can be any amino acid residue which is  
 different from the amino acid residue normally resident  
 at that position, and wherein the resulting antibody  
 binds selectively to an OPGbp.

The invention also provides for anti-OPGbp  
 20 antibodies comprising variable light and variable heavy  
 chains and further comprising a light chain CDR3  
 sequence which is increased from five amino acids to up  
 to nine amino acids. More particularly, the light  
 chain CDR3 sequence is selected from the group  
 25 consisting of:

QHTXXXXRA (SEQ ID NO: 97)

wherein the first occurrence of X from left to right  
 30 denotes any amino acid residue other than arginine, the  
 second, third and fourth occurrences of X denote any  
 amino acid residue, but preferably alanine, and wherein  
 the resulting antibody binds selectively to an OPGbp.  
 In another embodiment of the invention, a light chain  
 35 CDR3 sequence is selected from the group consisting of:

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QHTXAAARA (SEQ ID NO: 98)

wherein X is any amino acid residue other than arginine.

5 In another embodiment, the antibody variants of the invention comprise  $V_L$  chains having a CDR1 sequence as in SEQ ID NO:1 and a CDR2 sequence as in SEQ ID NO:5, and comprise  $V_H$  chains having  $V_H$  chains having a CDR1 sequence as in SEQ ID NO:13 and a CDR2  
10 sequence as in SEQ ID NO:16. In another embodiment, the antibody variants comprise a  $V_L$  chain from "AT" antibody with the aforementioned light chain CDR3 variants and a  $V_H$  chain from "AT" antibody with the aforementioned heavy chain CDR3 variants. Variants may  
15 also be prepared by "chain shuffling" of either light or heavy chains (Marks et al. Biotechnology 10, 779-783 (1992)). Typically, a single light (or heavy) chain is combined with a library having a repertoire of heavy (or light) chains and the resulting population is  
20 screened for a desired activity, such as binding to OPGbp. This technique permits screening of a greater sample of different heavy (or light) chains in combination with a single light (or heavy) chain than is possible with libraries comprising repertoires of  
25 both heavy and light chains.

The selective binding agents of the invention can be bispecific. Bispecific selective binding agents of this invention can be of several configurations. For example, bispecific antibodies resemble single  
30 antibodies (or antibody fragments) but have two different antigen binding sites (variable regions). Bispecific antibodies can be produced by chemical techniques (see e.g., Kranz et al., *Proc. Natl. Acad. Sci. USA*, 78:5807 (1981)), by "polydoma" techniques  
35 (see U.S. Pat. No. 4,474,893 to Reading) or by recombinant DNA techniques.

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The selective binding agents of the invention may also be heteroantibodies. Heteroantibodies are two or more antibodies, or antibody binding fragments (Fab) linked together, each antibody or fragment having a different specificity.

The invention also relates to "humanized" antibodies. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into a human antibody from a source which is non-human. In general, non-human residues will be present in CDRs. Humanization can be performed following methods known in the art (Jones et al., *Nature* 321, 522-525 (1986); Riechmann et al., *Nature*, 332, 323-327 (1988); Verhoeven et al., *Science* 239, 1534-1536 (1988)), by substituting rodent complementarily-determining regions (CDRs) for the corresponding regions of a human antibody.

The selective binding agents of the invention, including chimeric, CDR-grafted, and humanized antibodies can be produced by recombinant methods known in the art. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein and known in the art. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Fully human antibodies may be produced by expression of recombinant DNA transfected into host cells or by expression in hybridoma cells as described above.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. To do so, antibody-specific messenger RNA molecules are extracted from immune system cells taken



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from an immunized animal, and transcribed into complementary DNA (cDNA). The cDNA is then cloned into a bacterial expression system. One example of such a technique suitable for the practice of this invention  
5 uses a bacteriophage lambda vector system having a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great  
10 numbers of functional Fab fragments for those which bind the antigen. Such OPGbp selective binding agents (Fab fragments with specificity for an OPGbp polypeptide) are specifically encompassed within the term "antibody" as it is defined, discussed, and  
15 claimed herein.

Also within the scope of the invention are techniques developed for the production of chimeric antibodies by splicing the genes from a mouse antibody molecule of appropriate antigen-specificity together  
20 with genes from a human antibody molecule of appropriate biological activity, such as the ability to activate human complement and mediate ADCC. (Morrison et al., *Proc. Natl. Acad. Sci.*, 81:6851 (1984); Neuberger et al., *Nature*, 312:604 (1984)). One example  
25 is the replacement of a Fc region with that of a different isotype. Selective binding agents such as antibodies produced by this technique are within the scope of the invention.

In a preferred embodiment of the invention,  
30 the anti-OPGbp antibodies are fully human antibodies. Thus encompassed by the invention are antibodies which bind OPGbp polypeptides and are encoded by nucleic acid sequences which are naturally occurring somatic variants of human germline immunoglobulin nucleic acid  
35 sequence, and fragments, synthetic variants, derivatives and fusions thereof. Such antibodies may

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be produced by any method known in the art. Exemplary methods include immunization with a OPGbp antigen (any OPGbp polypeptide capable of eliciting an immune response, and optionally conjugated to a carrier) of transgenic animals (e.g., mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. See, for example, Jakobovits et al., *Proc. Natl. Acad. Sci.*, 90, 2551-2555 (1993); Jakobovits et al., *Nature*, 362, 255-258 (1993); Bruggermann et al., *Year in Immunol.*, 7, 33 (1993).

Alternatively, human antibodies may be generated through the in vitro screening of phage display antibody libraries. See Hoogenboom et al., *J. Mol. Biol.*, 227, 381 (1991); Marks et al., *J. Mol. Biol.*, 222, 581 (1991), incorporated herein by reference. Various antibody-containing phage display libraries have been described and may be readily prepared by one skilled in the art. Libraries may contain a diversity of human antibody sequences, such as human Fab, Fv, and scFv fragments, that may be screened against an appropriate target. Example 1 describes the screening of a Fab phage library against OPGbp to identify those molecules which selectively bind OPGbp. It will be appreciated that phage display libraries may comprise peptides or proteins other than antibodies which may be screened to identify selective binding agents of OPGbp.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the monoclonal antibody with the monoclonal antibody to which an anti-Id is being prepared. The immunized animal will recognize

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and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. Pat. No. 4,699,880, which is herein  
5 entirely incorporated by reference. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original monoclonal  
10 antibody which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

15 Production of selective binding agents of OPGbp

When the selective binding agent of OPGbp to be prepared is a proteinaceous selective binding agent, such as an antibody or an antigen binding domain, various biological or chemical methods for producing  
20 said agent are available.

Biological methods are preferable for producing sufficient quantities of a selective binding agent for therapeutic use. Standard recombinant DNA techniques are particularly useful for the production  
25 of antibodies and antigen binding domains of the invention. Exemplary expression vectors, host cells and methods for recovery of the expressed product are described below.

A nucleic acid molecule encoding an OPGbp  
30 antibody or antigen binding domain is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery  
35 such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule

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encoding an anti-OPGbp antibody may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether an anti-OPGbp antibody is to be post-  
5 transitionally modified (e.g., glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian host cells are preferable. For a review of expression vectors, see Meth. Enz. v. 185, D.V. Goeddel, ed.  
10 Academic Press Inc., San Diego, CA (1990)

Typically, expression vectors used in any host cells will contain one or more of the following components: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination  
15 sequence, a complete intron sequence containing a donor and acceptor splice site, a leader sequence for secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a  
20 selectable marker element. Each of these sequences is discussed in more detail below.

The vector components may be homologous (i.e., from the same species and/or strain as the host cell), heterologous (i.e., from a species other than  
25 the host cell species or strain), hybrid (i.e., a combination of different sequences from more than one source), synthetic, or native sequences which normally function to regulate immunoglobulin expression. As such, a source of vector components may be any  
30 prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the components are functional in, and can be activated by, the host cell machinery.

An origin of replication is selected based  
35 upon the type of host cell being used for expression. For example, the origin of replication from the plasmid

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pBR322 (Product No. 303-3s, New England Biolabs, Beverly, MA) is suitable for most Gram-negative bacteria while various origins from SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV) or papillomaviruses (such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding regions and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described above.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the



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process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the marker present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes an anti-OPGbp antibody. As a result, increased quantities of an antibody are synthesized from the amplified DNA.

A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of the polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth above and used in a prokaryotic vector.

A leader, or signal, sequence is used to direct secretion of a polypeptide. A signal sequence may be positioned within or directly at the 5' end of a polypeptide coding region. Many signal sequences have been identified and may be selected based upon the host cell used for expression. In the present invention, a signal sequence may be homologous (naturally occurring) or heterologous to a nucleic acid sequence encoding an



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anti-OPGbp antibody or antigen binding domain. A heterologous signal sequence selected should be one that is recognized and processed, i.e., cleaved, by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process a native immunoglobulin signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, a native immunoglobulin signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In most cases, secretion of an anti-OPGbp antibody or antigen binding domain from a host cell will result in the removal of the signal peptide from the antibody. Thus the mature antibody will lack any leader or signal sequence.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add presequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid found in the peptidase cleavage site, attached to the N-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired

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OPGbp polypeptide, if the enzyme cuts at such area within the mature polypeptide.

The expression vectors of the present invention will typically contain a promoter that is recognized by the host organism and operably linked to a nucleic acid molecule encoding an anti-OPGbp antibody or antigen binding domain. Either a native or heterologous promoter may be used depending the host cell used for expression and the yield of protein desired.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence(s), using linkers or adapters as needed to supply any required restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, e.g., heat-shock promoters and the actin promoter.

Additional promoters which may be used for expressing the selective binding agents of the invention include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, Nature, 290:304-310, 1981); the CMV promoter; the promoter

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contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., *Cell*, 22; 787-797, 1980); the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78; 144-1445, 1981); the regulatory sequences of the metallothioneine gene (Brinster et al., *Nature*, 296; 39-42, 1982); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Komaroff, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 75; 3727-3731, 1978); or the tac promoter (DeBoer, et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80; 21-25, 1983). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell*, 38; :639-646, 1984; Ornitz et al., *Cold Spring Harbor Symp. Quant. Biol.* 50; 399-409, 1986; MacDonald, *Hepatology*, 7; :425-515, 1987); the insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature*, 315; 115-122, 1985); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., *Cell*, 38; 647-658, 1984; Adames et al., *Nature*, 318; 533-538, 1985; Alexander et al., *Mol. Cell. Biol.*, 7; 1436-1444, 1987); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., *Cell*, 45; 485-495, 1986), albumin gene control region which is active in liver (Pinkert et al., *Genes and Devel.*, 1; 268-276, 1987); the alphafetoprotein gene control region which is active in liver (Krumlauf et al., *Mol. Cell. Biol.*, 5; 1639-1648, 1985; Hammer et al., *Science*, 235; 53-58, 1987); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., *Genes and Devel.*, 1; 161-171, 1987); the beta-globin gene control region which is active in myeloid cells (Mogam et al.,

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Nature, 315; 338-340, 1985; Kollias et al., Cell, 46; 89-94, 1986); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., Cell, 48; 703-712, 1987); the  
5 myosin light chain-2 gene control region which is active in skeletal muscle (Sani, Nature, 314; 283-286, 1985); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., Science, 234; 1372-1378, 1986).  
10 An enhancer sequence may be inserted into the vector to increase transcription in eucaryotic host cells. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically,  
15 however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer  
20 may be spliced into the vector at a position 5' or 3' to the polypeptide coding region, it is typically located at a site 5' from the promoter.

Preferred vectors for practicing this invention are those which are compatible with  
25 bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2  
30 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional possible vectors include, but are  
35 not limited to, cosmids, plasmids or modified viruses, but the vector system must be compatible with the

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selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript<sup>®</sup> plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO<sup>™</sup> TA Cloning<sup>®</sup> Kit, PCR2.1<sup>®</sup> plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, or other known techniques.

Host cells of the invention may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). The host cell, when cultured under appropriate conditions, expresses an antibody or antigen binding domain of the invention which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). Selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), Manassas, VA. Examples include mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR- cells (Urlaub et al. Proc. Natl. Acad. Sci. USA 97, 4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The



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selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the  
5 monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell  
10 strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines  
15 include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the American Type Culture Collection, Manassas, VA). Each of these cell  
20 lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101,  
25 (ATCC No. 33694) DH5 $\alpha$ , DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas* spp., other *Bacillus* spp., *Streptomyces* spp., and the like may also be employed in this method.

30 Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerevisiae*.



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Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts et al. (Biotechniques, 14, 810-817 (1993)),  
5 Lucklow (Curr. Opin. Biotechnol., 4, 564-572 (1993) and Lucklow et al. (J. Virol., 67, 4566-4579 (1993)). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

Transformation or transfection of a nucleic  
10 acid molecule encoding an anti-OPGbp antibody or antigen binding domain into a selected host cell may be accomplished by well known methods including methods such as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method.  
15 The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook et al., supra.

20 One may also use transgenic animals to express glycosylated selective binding agents, such as antibodies and antigen binding domain. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain glycosylated binding  
25 agents in the animal milk. Alternatively, one may use plants to produce glycosylated selective binding agents.

Host cells comprising (i.e., transformed or transfected) an expression vector encoding a selective  
30 binding agent of OPGbp may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells are for example, Luria Broth  
35 (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM,

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all of which may be supplemented with serum and/or growth factors as required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate,  
5 lactalbumin hydrolysate, and/or fetal calf serum as necessary.

Typically, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the  
10 media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium  
15 will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline and neomycin

The amount of an anti-OPGbp antibody or antigen binding domain produced by a host cell can be evaluated using standard methods known in the art.  
20 Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays.

Purification of an anti-OPG antibody or  
25 antigen binding domain which has been secreted into the cell media can be accomplished using a variety of techniques including affinity, immunoaffinity or ion exchange chromatography, molecular sieve chromatography, preparative gel electrophoresis or  
30 isoelectric focusing, chromatofocusing, and high pressure liquid chromatography. For example, antibodies comprising a Fc region may be conveniently purified by affinity chromatography with Protein A, which selectively binds the Fc region. Modified forms  
35 of an antibody or antigen binding domain may be prepared with affinity tags, such as hexahistidine or

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other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or myc (Invitrogen) at either its carboxyl or amino terminus and purified by a one-step affinity column. For example, polyhistidine binds with  
5 great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of polyhistidine-tagged selective binding agents. (See for example, Ausubel et al., eds., *Current Protocols in Molecular*  
10 *Biology*, Section 10.11.8, John Wiley & Sons, New York (1993)). In some instances, more than one purification step may be required.

Selective binding agents of the invention which are expressed in procaryotic host cells may be  
15 present in soluble form either in the periplasmic space or in the cytoplasm or in an insoluble form as part of intracellular inclusion bodies. Selective binding agents can be extracted from the host cell using any standard technique known to the skilled artisan. For  
20 example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

Soluble forms of an anti-OPGbp antibody or  
25 antigen binding domain present either in the cytoplasm or released from the periplasmic space may be further purified using methods known in the art, for example Fab fragments are released from the bacterial periplasmic space by osmotic shock techniques.

30 If an antibody or antigen binding domain has formed inclusion bodies, they can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be  
35 treated at pH extremes or with chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or

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urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The  
5 soluble selective binding agent can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate a solubilized antibody or antigen binding domain, isolation may be accomplished using standard methods such as those set  
10 forth below and in Marston et al. (*Meth. Enz.*, 182:264-275 (1990)).

In some cases, an antibody or antigen binding domain may not be biologically active upon isolation. Various methods for "refolding" or converting the  
15 polypeptide to its tertiary structure and generating disulfide linkages, can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a  
20 chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases  
25 the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s).  
30 Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol (DTT)/dithiane DTT, and 2-mercaptoethanol (BME)/dithio-b (ME). In many  
35 instances, a cosolvent may be used or may be needed to increase the efficiency of the refolding and the more common reagents used for this purpose include glycerol,

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polyethylene glycol of various molecular weights, arginine and the like.

Antibodies and antigen binding domains of the invention may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield et al., (*J. Am. Chem. Soc.*, 85:2149 [1963]), Houghten et al. (*Proc Natl Acad. Sci. USA*, 82:5132 [1985]), and Stewart and Young (*Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL [1984]). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized antibodies and antigen binding domains may be oxidized using methods set forth in these references to form disulfide bridges. Antibodies so prepared will retain at least one biological activity associated with a native or recombinantly produced anti-OPGbp antibody or antigen binding domain.

#### 20 Assays for selective binding agents of OPGbp

Screening methods for identifying selective binding agents which partially or completely inhibits at least one biological activity of OPGbp are provided by the invention. Inhibiting the biological activity of OPGbp includes, but is not limited to, inhibiting binding of OPGbp to its cognate receptor, ODAR, inhibiting stimulation of osteoclast formation in vitro or in vivo by OPGbp, and/or inhibiting bone turnover or bone resorption mediated by OPGbp. Selective binding agents of the invention include anti-OPGbp antibodies, and fragments, variants, derivatives and fusion thereof, peptides, peptidomimetic compounds or organo-mimetic compounds.

Screening methods for identifying selective binding agents which can partially or completely inhibit a biological activity of OPGbp can include in



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vitro or in vivo assays. In vitro assays include those that detect binding of OPGbp to ODAR and may be used to screen selective binding agents of OPGbp for their ability to increase or decrease the rate or extent of OPGbp binding to ODAR. In one type of assay, an OPGbp polypeptide, preferably a soluble form of OPGbp such as an extracellular domain, is immobilized on a solid support (e.g., agarose or acrylic beads) and an ODAR polypeptide is added either in the presence or absence of a selective binding agent of OPGbp. The extent of binding of OPGbp and ODAR with or without a selective binding agent present is measured. Binding can be detected by for example radioactive labeling, fluorescent labeling or enzymatic reaction.

Alternatively, the binding reaction may be carried out using a surface plasmon resonance detector system such as the BIAcore assay system (Pharmacia, Piscataway, NJ). Binding reactions may be carried out according to the manufacturer's protocol.

In vitro assays such as those described above may be used advantageously to screen rapidly large numbers of selective binding agents for effects on binding of OPGbp to ODAR. The assays may be automated to screen compounds generated in phage display, synthetic peptide and chemical synthesis libraries.

Selective binding agents increase or decrease binding of OPGbp to ODAR may also be screened in cell culture using cells and cell lines expressing either polypeptide. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. As an example, the binding of OPGbp to cells expressing ODAR on the surface is evaluated in the presence or absence of selective binding agents and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to OPGbp.



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In vitro activity assays may also be used to identify selective binding agents which inhibit OPGbp activity. Examples of assays include stimulation of cell growth and proliferation which are dependent on OPGbp and OPGbp mediated osteoclast formation from bone marrow cells, the latter of which is described in Example 1 of the present application.

In vivo assays are also available to determine whether a selective binding agent is capable of decreasing or inhibiting bone turnover and/or bone resorption. Bone resorption can be increased in animals by a variety of methods, including ovariectomy and administration of pro-resorptive agents such as OPGbp or IL-1. See WO 97/23614 and WO 98/46751. The effects of OPG inhibitors on bone resorption in human patients may be measured by a variety of known methods such as single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT), and ultrasonography (See Johnston et al. in Primer on the Metabolic Bone Disease and Disorders of Mineral Metabolism, 2<sup>nd</sup> ed., M.J. Favus, ed. Raven Press pp. 137-146). Bone turnover and resorption may also be determined by measuring changes in certain biochemical markers, such as serum osteocalcin, serum alkaline phosphatase, serum procollagen I extension peptides, urinary or serum C-terminal or N-terminal telopeptide of collagen, urinary calcium, hydroxyproline and urinary pyridinoline and deoxypyridinoline. It is generally recognized that a decrease in the levels of the aforementioned biochemical markers indicates that bone resorption is decreased and loss of bone mass is being reduced. Alternatively, effects on bone resorption may also be determined by measuring a change in the mechanical strength of bone, in particular an increase in torsional (twisting) strength of bone.

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For diagnostic applications, in certain embodiments, selective binding agents of OPGbp, such as antibodies and antigen binding domains thereof, typically will be labeled with a detectable moiety.

5 The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as <sup>3</sup>H, <sup>14</sup>C, <sup>32</sup>P, <sup>35</sup>S, or <sup>125</sup>I, a fluorescent or chemiluminescent compound, such as  
10 fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase,  $\beta$ -galactosidase or horseradish peroxidase. Bayer et al., *Meth. Enz.*, 184: 138-163 (1990).

The selective binding agents of the invention  
15 may be employed in any known assay method, such as radioimmunoassays, competitive binding assays, direct and indirect sandwich assays (ELISAs), and immunoprecipitation assays (Sola, *Monoclonal Antibodies: A Manual of Techniques*, pp. 147-158 (CRC  
20 Press, 1987)) for detection and quantitation of OPGbp polypeptides. The antibodies will bind OPGbp polypeptides with an affinity which is appropriate for the assay method being employed.

The selective binding agents of the invention  
25 also are useful for *in vivo* imaging, wherein for example a selective binding agent labeled with a detectable moiety is administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is  
30 assayed. The agent may be labeled with any moiety that is detectable in an animal; whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

The invention also relates to a kit  
35 comprising a selective binding agent of OPGbp, such as

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an antibody or antigen binding domain, and other reagents useful for detecting OPGbp levels in biological samples. Such reagents may include a secondary activity, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

#### Therapeutic Uses of OPGbp selective binding agents

Selective binding agents of the invention may be used as therapeutics. Therapeutic selective binding agents may be OPGbp agonists or antagonists and, in one embodiment, are anti-OPGbp antagonist antibodies which inhibit at least one of the biological activities of a OPGbp polypeptide in vitro or in vivo. For example, an antagonist of OPGbp will inhibit the binding of OPGbp to ODAR by at least about 100-fold, or about 1000-fold, or greater than 1000-fold. Alternatively, an OPGbp antagonist will inhibit osteoclast formation in vitro as indicated by a measurable IC50 (a concentration giving 50% inhibition) in a bone marrow assay such as that described in Example 1. Alternatively, an OPGbp antagonist will decrease bone turnover markers by at least 20%, or at least 50% compared to baseline levels. Antagonist OPGbp selective binding agents (such as antibodies) are identified by screening assays described herein.

OPGbp antagonists, such as anti-OPGbp antagonist antibodies and antigen binding domains, may be used to prevent or treat bone diseases characterized by loss of bone mass or by replacement of structurally normal bone with structurally abnormal bone. OPGbp antagonists may be administered to an animal having loss of bone mass or susceptible to having loss of bone mass resulting from any of the following disorders: Osteoporosis, such as primary osteoporosis, endocrine osteoporosis (hyperthyroidism, hyperparathyroidism,

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Cushing's syndrome, and acromegaly), hereditary and congenital forms of osteoporosis (osteogenesis imperfecta, homocystinuria, Menkes' syndrome, and Riley-Day syndrome) and osteoporosis due to

5 immobilization of extremities; Osteomyelitis, or an infectious lesion in bone, leading to loss of bone mass; Hypercalcemia resulting from solid tumors (breast, lung and kidney) and hematologic malignancies (multiple myeloma, lymphoma and leukemia), idiopathic

10 hypercalcemia, and hypercalcemia associated with hyperthyroidism and renal function disorders; Osteopenia following surgery, induced by steroid administration, and associated with disorders of the small and large intestine and with chronic hepatic and

15 renal diseases; Osteonecrosis, or bone cell death, associated with traumatic injury or nontraumatic necrosis associated with Gaucher's disease, sickle cell anemia, systemic lupus erythematosus and other conditions; Loss of bone mass due to rheumatoid

20 arthritis; Periodontal loss of bone mass; Osteoarthritis; Prosthetic loosening; and Osteolytic metastasis. OPGbp antagonists may also be used to prevent or treat certain bone disorders are characterized by the replacement of structurally sound

25 bone with disorganized structurally incompetent bone, such as Paget's disease of bone (osteitis deformans) in adults and juveniles; hyperparathyroidism, in congenital bone disorders such as fibrous dysplasia, and in osteosclerotic bone metastases.

30 In an embodiment of the invention, OPGbp antagonists are advantageously used to treat loss of bone mass resulting from osteolytic destruction of bone caused by malignant or metastatic tumors. OPG polypeptides of the invention may be used to treat loss

35 of bone mass associated with breast, prostate, thyroid, kidney, lung, esophageal, rectal, bladder, cervical,

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ovarian and liver cancers as well as cancer of the gastrointestinal tract. Also included is loss of bone mass associated with certain hematological malignancies such as multiple myeloma and lymphomas such as

5 Hodgkin's Disease.

OPGbp antagonists of the invention, including antagonist antibodies and antigen binding domains, are administered alone or in combination with other therapeutic agents, in particular, in combination with

10 other cancer therapy agents. Such agents generally include radiation therapy or chemotherapy. Chemotherapy may involve treatment with one or more of the following: anthracyclines, taxol, tamoxifene, doxorubicin, 5-fluorouracil, and other drugs known to

15 the skilled worker. In one embodiment, the cancer therapy agent is a luteinizing hormone-releasing hormone (LHRH) antagonist, preferably a peptide antagonist. More preferably, an LHRH antagonist is a decapeptide comprising the following structure:

20 A-B-C-D-E-F-G-H-I-J

wherein

A is pyro-glu, Ac-D-Nal, Ac-D-Qal; Ac-Sar, or Ac-D-Pal;

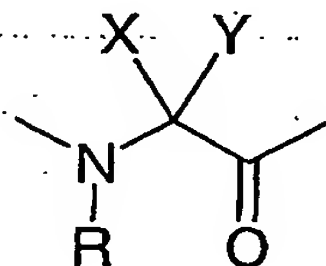
B is His or 4-Cl-D-Phe;

25 C is Trp, D-Pal, D-Nal, L-Nal-D-Pal(N-O), or D-Trp;

D is Ser;

E is N-Me-Ala, Tyr, N-Me-Tyr, Ser, Lys(iPr), 4-Cl-Phe, His, Asn, Met, Ala, Arg or Ile;

30 F is



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wherein R and X are independently, H and alkyl;  
and Y comprises a small polar entity.

G is Leu or Trp;

H is Lys(iPr), Gln, Met, or Arg;

5 I is Pro; and

J is Gly-NH<sub>2</sub> or D-Ala-NH<sub>2</sub>;

or a pharmaceutically acceptable salt thereof.

In another embodiment, an LHRH antagonist  
comprises the peptide:

10 N-Ac-D-Nal-4-Cl-Phe-D-Pal-Ser-N-Me-Tyr-D-Asn-Leu-  
Lys(iPr)-Pro-D-Ala-NH<sub>2</sub>.

Standard abbreviations and conventions are  
used herein and the following non-standard residues and  
moieties are abbreviated as follows:

15

Nal	3-(2-naphthyl)alaninyl
4-Cl-Phe	(4'-chlorophenyl)alaninyl
Pal	3-(3'-pyridyl)alaninyl
Pal(N-O)	3-(3'-pyridine-N-oxide)alaninyl
20 iPr-Lys	N-epsilon-2-propyl-lysiny
Qal	3-(2'-quinolinyl)alaninyl

Alternative forms of LHRH antagonist  
decapeptides are also encompassed by the invention.  
Such decapeptides are described in U.S. Patent No.  
25 5,843,901 hereby incorporated by reference.

Also included are combinations of OPGbp  
antagonists with antibodies which bind to tumor cells  
and induce a cytotoxic and/or cytostatic effect on  
tumor growth. Examples of such antibodies include  
30 those which bind to cell surface proteins Her2, CDC20,  
CDC33, mucin-like glycoprotein and epidermal growth  
factor receptor (EGFR) present on tumor cells and  
induce a cytostatic and/or cytotoxic effect on tumor  
cells displaying these proteins. Examples of such  
35 antibodies include HERCEPTIN for treatment of breast  
cancer and RITUXAN for the treatment of non-Hodgkin's



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lymphoma. Also included as cancer therapy agents are polypeptides which selectively induce apoptosis in tumor cells, such as the TNF-related polypeptide TRAIL. OPGbp antagonists may be administered prior to, concurrent with, or subsequent to treatment with a cancer therapy agent. OPGbp antagonists may be administered prophylactically to prevent or mitigate the onset of loss of bone mass by metastatic cancer or may be given for the treatment of an existing condition of loss of bone mass due to metastasis.

OPGbp antagonists of the invention may be used to prevent and/or treat the growth of tumor cells in bone. Cancer which metastasizes to bone can spread readily as tumor cells stimulate osteoclasts to resorb the internal bone matrix. Treatment with an OPGbp antagonist will maintain bone density by inhibiting resorption and decrease the likelihood of tumor cells spreading throughout the skeleton. Any cancer which metastasizes to bone may be prevented and/or treated with an OPGbp antagonist.

In one embodiment, multiple myeloma may be prevented and/or treated with an OPGbp antagonist, such as an antibody. Multiple myeloma is localized to bone and affected patients typically exhibit a loss of bone mass due to increased osteoclast activation in localized regions. Myeloma cells either directly or indirectly produce OPGbp, which in turn activates osteoclasts resulting in local bone lysis surrounding the myeloma cells embedded in bone marrow spaces. The normal osteoclasts adjacent to the myeloma cell in turn produce IL-6, leading to growth and proliferation of myeloma cells. Myeloma cells expand in a clonal fashion and occupy bone spaces that are being created by inappropriate bone resorption. Treatment of an animal with an OPGbp antagonist blocks activation of osteoclasts which in turn leads to a decrease in IL-6

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production by osteoclasts, and a suppression of myeloma all growth and/or proliferation.

OPGbp antagonists may be used alone for the treatment of the above referenced conditions resulting in loss of bone mass or in combination with a therapeutically effective amount of a bone growth promoting (anabolic) agent or a bone anti-resorptive agent including bone morphogenic factors designated BMP-1 to BMP-12, transforming growth factor- $\beta$  and TGF- $\beta$  family members, fibroblast growth factors FGF-1 to FGF-10, interleukin-1 inhibitors, TNF $\alpha$  inhibitors, parathyroid hormone, E series prostaglandins, bisphosphonates and bone-enhancing minerals such as fluoride and calcium. Anabolic agents include parathyroid hormone and insulin-like growth factor (IGF), wherein the latter agent is preferably complexed with an IGF binding protein. Preferred embodiments also include the combination of an OPGbp antagonist with a interleukin-1 (IL-1) receptor antagonist or an OPGbp antagonist with a soluble TNF receptor, such as soluble TNF receptor-1 or soluble TNF receptor-2. An exemplary IL-1 receptor antagonist is described in WO89/11540 and an exemplary soluble TNF receptor-1 is described in WO98/01555.

A decrease in the rate of bone resorption can lead to osteopetrosis, a condition marked by excessive bone density. Agonists of OPGbp may increase osteoclast formation and bone resorption and be administered to an animal which has or is susceptible to decreased bone resorption and an abnormal increase in bone mass.

#### Pharmaceutical Compositions

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Pharmaceutical compositions of OPGbp selective binding agents are within the scope of the present invention. Such compositions comprise a therapeutically or prophylactically effective amount of an OPGbp selective binding agent such as an antibody, or a fragment, variant, derivative or fusion thereof, in admixture with a pharmaceutically acceptable agent. In a preferred embodiment, pharmaceutical compositions comprise anti-OPGbp antagonist antibodies which inhibit partially or completely at least one biological activity of OPGbp in admixture with a pharmaceutically acceptable agent. Typically, the antibodies will be sufficiently purified for administration to an animal.

Pharmaceutically acceptable agents for use in the compositions of the invention include carriers, excipients, diluents, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials and surfactants, as are well known in the art.

Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. Also included in the compositions are antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol. Also by way of example, suitable

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tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol and the like. Suitable preservatives include, but are not limited to, benzalkonium chloride, 5 thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide may also be used as preservative. Suitable cosolvents are for example glycerin, propylene glycol, and polyethylene glycol. Suitable complexing 10 agents are for example caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxy-propyl-beta-cyclodextrin. Suitable surfactants or wetting agents include sorbitan esters, polysorbates such as polysorbate 80, tromethamine, lecithin, cholesterol, tyloxapal and the 15 like. The buffers can be conventional buffers such as acetate, borate, citrate, phosphate, bicarbonate, or Tris-HCl. Acetate buffer may be around pH 4.0-5.5 and Tris buffer may be around pH 7.0-8.5. Additional pharmaceutical agents are set forth in *Remington's* 20 *Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company 1990, the relevant portions of which are hereby incorporated by reference.

The compositions may be in liquid form or in a lyophilized or freeze-dried form. Lyophilized forms 25 may include excipients such as sucrose.

The compositions of the invention are suitable for parenteral administration. In preferred embodiments, the compositions are suitable for injection or infusion into an animal by any route 30 available to the skilled worker, such as subcutaneous, intravenous, intramuscular, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, or intralesional routes. A parenteral 35 formulation will typically be a sterile, pyrogen-free,

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isotonic aqueous solution, optionally containing pharmaceutically acceptable preservatives.

The optimal pharmaceutical formulation may be readily determined by one skilled in the art depending upon the intended route of administration, delivery format and desired dosage.

Other formulations are also contemplated by the invention. The pharmaceutical compositions also may include particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or the introduction of an OPGbp selective binding agent (such as an antibody) into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation.

Pharmaceutical compositions also include the formulation of OPGbp selective binding agents (such as antibodies) with an agent, such as injectable microspheres, bio-erodible particles or beads, or liposomes, that provides for the controlled or sustained release of a selective binding agent which may then be delivered as a depot injection. Other suitable means for delivery include implantable delivery devices.

A pharmaceutical composition comprising and OPGbp selective binding agent (such as an antibody) may be formulated as a dry powder for inhalation. Such inhalation solutions may also be formulated in a liquefied propellant for aerosol delivery. In yet another formulation, solutions may be nebulized.

It is also contemplated that certain formulations containing OPGbp-selective binding agents may be administered orally. Formulations administered in this fashion may be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the



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active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized.

Additional agents may be included to facilitate  
5 absorption of a selective binding agent. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Another preparation may involve an effective  
10 quantity of an OPGbp selective binding agent in a mixture with non-toxic excipients which are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit dose form.  
15 Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium  
20 stearate, stearic acid, or talc.

Additional formulations will be evident to those skilled in the art, including formulations involving OPGbp selective binding agents in combination with one or more other therapeutic agents. Techniques  
25 for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See, for example, the Supersaxo et al. description of  
30 controlled release porous polymeric microparticles for the delivery of pharmaceutical compositions (See WO 93/15722 (PCT/US93/00829) the disclosure of which is hereby incorporated by reference.

Regardless of the manner of administration,  
35 the specific dose may be calculated according to body weight, body surface area or organ size. Further



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refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the  
5 ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

One may further administer the present pharmaceutical compositions by pulmonary  
10 administration, see, e.g., PCT W094/20069, which discloses pulmonary delivery of chemically modified proteins, herein incorporated by reference. For pulmonary delivery, the particle size should be suitable for delivery to the distal lung. For example,  
15 the particle size may be from 1 $\mu$ m to 5 $\mu$ m, however, larger particles may be used, for example, if each particle is fairly porous.

Alternatively or additionally, the compositions may be administered locally via  
20 implantation into the affected area of a membrane, sponge, or other appropriate material on to which an OP or an OPGbp selective binding agent has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or  
25 organ, and delivery of an OPGbp selective binding agent may be directly through the device via bolus, or via continuous administration, or via catheter using continuous infusion.

Pharmaceutical compositions of the invention  
30 may also be administered in a sustained release formulation or preparation. Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices  
35 include polyesters, hydrogels, polylactides (See e.g.,

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U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al, *Biopolymers*, 22: 547-556 [1983]), poly (2-hydroxyethyl-methacrylate) (Langer et al., *J. Biomed. Mater. Res.*, 15: 167-277 [1981] and Langer, *Chem. Tech.*, 12: 98-105 [1982]), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art.

See e.g., Eppstein et al., *Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 (1985); EP 36,676; EP 88,046; and EP 143,949.

OPGbp selective binding agents, such as antibodies and fragments, variants, derivatives and fusions thereof, may be employed alone or in combination with other pharmaceutical compositions. For example, pharmaceutical compositions comprising separately or together an OPGbp antagonist and an interleukin-1 receptor antagonist, or an OPGbp antagonist and a soluble TNF receptor-1, or an OPGbp antagonist and a soluble TNF receptor-2 may be used for the treatment of rheumatoid arthritis. Further, compositions comprising separately or together an OPGbp antagonist and a cancer therapy agent may be used for the treatment of cancer and associated loss of bone mass. Other combinations with an OPGbp antagonist or agonist are possible depending upon the condition being treated.

It may be desirable in some instances to use a pharmaceutical composition comprising an OPGbp selective binding agent compositions in an ex vivo manner. Here, cells, tissues, or organs that have been removed from the patient are exposed to pharmaceutical compositions comprising OPGbp selective binding agents after which the cells, tissues and/or organs are subsequently implanted back into the patient.

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In other cases, a composition comprising an OPGbp selective binding agent may be delivered through implanting into patients certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptides, selective binding agents, fragments, variants, or derivatives. Such cells may be animal or human cells, and may be derived from the patient's own tissue or from another source, either human or non-human. Optionally, the cells may be immortalized. However, in order to decrease the chance of an immunological response, it is preferred that the cells be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow release of the protein product(s) but prevent destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Methods used for membrane encapsulation of cells are familiar to the skilled artisan, and preparation of encapsulated cells and their implantation in patients may be accomplished without undue experimentation. See, e.g., U.S. Patent Nos. 4,892,538; 5,011,472; and 5,106,627. A system for encapsulating living cells is described in PCT WO 91/10425 (Aebischer et al.). Techniques for formulating a variety of other sustained or controlled delivery means, such as liposome carriers, bio-erodible particles or beads, are also known to those in the art, and are described. The cells, with or without encapsulation, may be implanted into suitable body tissues or organs of the patient.

A therapeutically or prophylactically effective amount of a pharmaceutical composition comprising an OPGbp selective binding agent (such as an

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anti-OPGbp antibody, or fragment, variant, derivative, and fusion thereof) will depend, for example, upon the therapeutic objectives such as the indication for which the composition is being used, the route of administration, and the condition of the subject.

OPGbp antagonist antibodies or antigen binding domains of the invention are administered in a therapeutically or prophylactically effective amount to prevent and/or treat loss of bone associated with metastatic bone disease. A "therapeutically or prophylactically effective amount" of an OPGbp antagonist antibody is that amount which reduces the rate and/or extent of loss of bone mass or prevents the loss of bone mass in a subject having normal bone mass. Changes in bone mass are detected by a variety of known methods such as single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT), and ultrasonography (See Johnston et al. in Primer on the Metabolic Bone Disease and Disorders of Mineral Metabolism, 2<sup>nd</sup> ed., M.J. Favus, ed. Raven Press pp. 137-146). One skilled in the art can use these methods to determine a therapeutically effective amount of an OPG fusion polypeptide. A therapeutically effective amount may also be determined by measuring changes in biochemical markers for bone turnover, such as serum osteocalcin, serum alkaline phosphatase, serum procollagen I extension peptides, urinary or serum C-terminal or N-terminal telopeptide of collagen, urinary calcium, hydroxyproline and urinary pyridinoline and deoxypyridinoline. It is generally recognized that a decrease in the levels of the aforementioned biochemical markers indicates that bone resorption is decreased and loss of bone mass is being reduced.

Alternatively, a therapeutically effective amount of an OPG fusion polypeptide may also be determined by

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measuring a change in the mechanical strength of bone, in particular an increase in torsional (twisting) strength of bone.

Accordingly, it may be necessary for the caretaker to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1  $\mu\text{g/kg}$  to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 1  $\mu\text{g/kg}$  up to about 100 mg/kg; or 5  $\mu\text{g/kg}$  up to about 100 mg/kg; or 0.1  $\mu\text{g/kg}$  up to about 100 mg/kg; or 1  $\mu\text{g/kg}$  up to about 100 mg/kg. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of an OPGbp selective binding agent) over time, or as a continuous infusion via implantation device or catheter.

The following examples are offered to more fully illustrate the invention but are not construed as limiting the scope thereof.

#### Example 1

##### Reagents and Assays

The screening target used in these studies was prepared from expression of a cDNA encoding human OPGbp of amino acids 140 through 317 inclusive as shown in Figure 4 of PCT W098/46751 in a CHO host cell and

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purified as follows. A Q Sepharose column (Pharmacia) was equilibrated with 20mM tris pH 8.5. Conditioned media which had also been titrated to pH 8.5 was applied, the column washed with the Tris buffer, and proteins were eluted with a 100-600mM NaCl gradient over 20 column volumes. Fractions containing OPGL were identified through SDS-PAGE and Western blot analysis. OPGBP containing fractions were then titrated to pH 4.8 and applied to a Sp column (Pharmacia) which had been equilibrated with 20mM sodium acetate pH 4.8. After washing, proteins were eluted with a 0-0.3M NaCl gradient followed by 0.5M and 1M NaCl steps. OPGBP eluted with all buffers however only the 0-0.3M NaCl gradient fractions were found to be active in vitro osteoclast stimulating bioassays. The yield was 40mg/l. Amino-terminal sequencing revealed that about 80% of the purified protein started with amino acid 143 of human OPGBP while the remaining about 20% started with amino acid 147. The final product used for screening phage libraries is referred to as OPGBP[143-317], the predominant purified form.

Anti-OPGBP polyclonal antibodies were prepared as follows. Three white New Zealand rabbits (Western Oregon Rabbit Co., Philomath, OR) were initially injected with equal amounts of Hunter Titer Max (CytRx Corp., Atlanta, GA) and OPGBP[143-317]. 0.2 mgs per rabbit was injected. This was repeated four and six weeks later. A 50 ml bleed was performed at seven weeks and once per week thereafter for a total of six bleeds. The antibodies were affinity purified from sera of immunized rabbits on an OPGBP resin as follows. Three mls of Actigel Ald resin (Sterogene) were added to a 10ml column (Kontes Flex Colum) and washed with 50 mls of PBS. Three mgs of OPGBP[143-317] diluted into 3 mls of PBS was added to the Actigel column and shaken gently to mix. 0.6mls of 1M Na Cyanoborohydride was



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then added and the mixture shaken overnight at 4°C. The column was washed with 50mls of Pierce Gentle Elution Buffer (Pierce) followed by 150mls of PBS. 50mls of sera from immunized rabbits was sterile  
5 filtered through a 0.45µm filter, added to the column and the mixture was shaken overnight at 4°C. The following day the column contents were allowed to settle and the liquid phase was drained. The column was then washed with 150mls of PBS to an OD<sub>280</sub> of 0.002.  
10 Pierce Gentle Elution Buffer with 1% Glacial Acetic Acid was then added to the column and 1 ml fractions were collected at 10 min intervals and analyzed by OD<sub>280</sub>. Fractions containing the highest amount of OD<sub>280</sub> absorbing material were pooled and dialyzed against two  
15 liters of PBS for 48 hours. There was one buffer change during this time.

ELISA assays were performed on eluted phage pools by plating OPGbp[143-317] at 1.5 µg/ml in PBS pH 8.0 for 2 h at room temperature in Nunc Maxisorp  
20 Immunoplates on a rocker. A rinse solution of 2% MPBS (Block Buffer ) was added to the immunoplates, incubated for 3 min at room temperature and discarded. Blocking was performed for 1 hour at room temperature with 2% MPBS. Washes were performed 5X using TBS-  
25 Tween-20 (0.1%) (TBS; Tris Buffered Saline; 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl). A titration of phage were added using a minimum of 10<sup>10</sup> phage/well in Conjugate Dilution Buffer (0.4% Nonfat Dry Milk in TBS or, 0.4% M-TBS) for 1 hour at room temperature. Washes  
30 were performed using TBS-Tween-20 (0.1%). Anti-M13-horse radish peroxidase (HRP) Monoclonal Antibody Conjugate (Pharmacia Piscataway, NJ) was used at a 1/2000 dilution in 0.4% MTBS for 1.5 h at room temperature. Washes were performed 5 times with TBS-  
35 Tween-20 (0.1%). 2,2'-Azinobis(3-ethylbenzthiazoline-

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sulfonic acid) (ABTS) (Pierce, Rockford, IL) a colorimetric substrate for detection at OD405 was added. Positive controls for the detection of plated huOPG bp [143-317] was performed by addition of OPG[22-194]-Fc, followed by anti-Fc-alkaline phosphatase and para-nitrophenylphenol (pNPP) substrate for detection.

PCR conditions and 2xTY-AG culture

A typical polymerase chain reaction (PCR) was performed in a 96-well Thermowell plate. Each well contains 20  $\mu$ l of PCR reaction mix [2  $\mu$ l 10X PCR buffer (Gibco BRL Products, Grand Island, NY), 17.3  $\mu$ l water, 0.2  $\mu$ l dNTPs (25 mM), 0.2  $\mu$ l Primer 870-02, 0.2  $\mu$ l Primer 2182-83 (primer stocks 10 pmol/ $\mu$ l for insert amplification), 0.1  $\mu$ l Taq polymerase]. Individual colonies were picked and resuspended in a well and overlaid with 20  $\mu$ l of mineral oil, sealed, then placed in PCR machine.

870-02      5'-CCG ACT TTG CAC CTA GTT (SEQ ID NO: 22)  
2182-83      5'-TTT GTC GTC TTT CCA GAC GTT AGT (SEQ ID NO: 23)

A duplicate plate for preparing cultures was generated by transferring the same picked colony to the corresponding well position in a second 96-deep well block. Cultures were grown in 0.3 to 1.0 ml 2xTY-AG (2xTY broth: (16 g bacto-tryptone / liter water, 10 g Yeast extract / liter water, 5 g NaCl/ liter water), containing 100  $\mu$ g/ml ampicillin and 2% glucose). The block was sealed with air-permeable tape, centrifuged at 1000 rpm for 2 minutes to bring down the liquid, and 37°C incubator at 300 to 350 rpm overnight for culturing. The overnight cultures received 150  $\mu$ l/well of 50% glycerol, were mixed, and frozen at -80°C.

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The PCR reaction conditions were 40 cycles of 45 sec. at 90°C, 45 sec at 55°C, 1.5 min at 72°C, followed by a 72°C extension for 10 min. After the PCR reaction was complete, 2.5 to 4.0  $\mu$ l were run on 25-well 1% agarose gels with 0.5  $\mu$ l/ml ethidium bromide, using DNA molecular weight standards (Gibco BRL Products, Grand Island, NY, or Stratagene, La Jolla, CA) for 90 min at 90 volts. Only full-length inserts of greater than 1.6 kb were considered.

10 A 16  $\mu$ l aliquot of the PCR reactions was BstNI digested 3 hours at 60°C with a 30  $\mu$ l total digestion mixture containing 10  $\mu$ l water, 3  $\mu$ l of 10X REact Buffer 2 (GIBCO BRL Products), 0.3  $\mu$ l BSA (10 mg/ml), 0.7  $\mu$ l BstNI (GIBCO; 10,000 units/ml).  
15 Digested samples were run on a 25-well 3% agarose gels for 3.5 hours at 80 volts.

#### RAW cell assay

Varying concentrations of Fab test samples were  
20 mixed with a constant amount of human OPGbp[143-317] and incubated for at least one hour at room temperature in DMEM, 10% fetal bovine serum and 1x glutamine-penicillin-streptomycin mixture. The concentrations of Fab samples and OPGbp are indicated for each  
25 experiment. After incubation, the mixture was added to  $2 \times 10^4$  RAW 264.7 cells/well (American Type Culture Collection, Manassas, VA, Accession No. TIB-71) in a 96 well flat bottom tissue culture plate. RAW cells were cultured in DMEM with 10% fetal bovine serum and 1x  
30 glutamine-penicillin-streptomycin. After three days at 37°C and 5%CO<sub>2</sub>, the media was aspirated from the wells and the cells were stained for Tartrate Resistant Acid Phosphatase (TRAP), an osteoclast differentiation marker, by addition of 100  $\mu$ l per well of 0.1M citrate  
35 buffer with 0.1% Triton X-100, incubation for five minutes at room temperature, addition of para-

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nitrophenylphosphate (pNPP) substrate and tartrate in citrate buffer containing Triton X-100 (substrate concentration was 20 mM pNPP and 335 mM tartrate) and incubation for an additional five minutes at room  
5 temperature. The reaction was stopped by addition of NaOH to a concentration of 0.05M. Acid phosphatase converts the pNPP substrate to para-nitrophenol which is detected by absorbance at 405 nm. The change in absorbance at 405 nm was plotted as a function log dose  
10 for both controls and test samples. An analysis of Variance (ANOVA) and relative potency with 95% confidence limits was calculated. Positive controls included varying concentrations of OPG[22-194]-Fc fusion protein or an anti-OPGbp polyclonal antibody  
15 preparation preincubated with OPGbp[143-317] and incubated with RAW264.7 cells as described above.

#### Bone Marrow Assay

A murine bone marrow assay for osteoclast  
20 formation was carried out essentially as described in Lacey et al. (Cell 93, 165-176 (1998)) and Kong et al. (Nature 397, 315-323 (1999)). Briefly, the assay is a modification of the murine bone marrow coculture assay described in PCT WO97/23614 in which non-adherent  
25 murine bone marrow cells were cultured in media for about seven days in the presence of human OPGbp (143-317) but without addition of the stromal cell line ST2, 1,25(OH)<sub>2</sub> vitamin D3 and dexamethsone. Cells having an osteoclast phenotype were detected by the appearance of  
30 TRAP-positive cells. TRAP activity was measured in solution or by histochemical staining.

For detection of TRAP activity in solution, adult bone marrow cells were lysed in 100 mM Citrate Buffer (Sigma, Cat # 91-5) + 0.1% Triton X-100, pH 5.0,  
35 3-5 min. 20 mM pNPP, 80 mM Tartrate, and 100 mM Citrate + 0.1% Triton X-100, pH 5.0 were added and

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incubated at RT, 3-5 min, and measured at 405 nm after stopping the reaction with 50  $\mu$ l of 500 mM NaOH /well. A positive response was a concentration dependent decrease in absorbance at 405 nm from ~2.0 OD to ~0.6 OD.

For histochemical staining, cells were fixed in a formaldehyde-based fixative solution, then stained with Fast Garnet GBC (2-methyl-4-[(2-methylphenyl)-azo]benzenediazonium) solution + Naphthol AS-BI (C<sub>18</sub>H<sub>15</sub>BrNO<sub>6</sub>P) phosphate solution + Acetate Solution + Tartrate Solution, incubated 1 hour at 37°C, then rinsed, dried, and evaluated microscopically. A cell that was TRAP positive and contained three or more nuclei (TRAP-positive MNC) was considered an osteoclastic cell.

#### Example 2

##### Screening of a Human Fab library

A library of about 4 x10<sup>10</sup> unique human Fab fragments prepared in bacteriophage M13 was obtained from Target Quest, NV (Amsterdam, Netherlands). General procedures for construction and screening human Fab libraries were described in de Haard et al. (Advanced Drug Delivery Reviews 31, 5-31 (1998); J. Biol. Chem. 274, 18218-18230 (1999)). The library was screened for Fab fragments which bind to OPGbp[143-317] by the following procedures.

##### Solid phase direct plating

OPGbp [143-317] prepared as described above was immobilized on a solid phase using Nunc Maxisorb immunotubes (12 x 75mm, 5ml capacity) by directly plating on the solid phase at a protein concentration

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of 1.5 µg/ml in TBS, pH 8.0 (TBS was Tris buffered saline: 10 mM Tris (pH 7.5), 150 mM NaCl) at room temperature for 2 hours. These conditions permitted 80% of maximum plating of the solid phase at 2 hours (maximum at 2 hours was still nonsaturating) while retaining binding capabilities to OPG [22-194]-Fc. After the 2 hour incubation, the tube was washed three times with PBS. The plated target was blocked by filling the immunotube with 2% nonfat dry milk, (Marvel or Carnation) in PBS (MPBS) for 1 to 4 hours at room temperature, washed two times each in PBS-Tween 20 (0.1%) and PBS. The PEG-concentrated phage (approximately  $10^{13}$ ) were pre-blocked in 2% MPBS to adsorb milk binding phage prior to exposure of the phage to the solid phase target. The pre-blocked phage were incubated in 4 ml with the plated target at room temperature for 2 h, (30 min rotating end-over-end and 90 min standing). The contents bound to the tube were washed 20 times with PBS-Tween 20 (0.1%) and 20 times with PBS to remove unbound phage and to reduce nonspecific binding. Phage were eluted from the solid phase by a ten minute total phage elution with 1 ml of 100 mM triethylamine (TEA) pH 12, rotating the tube end-to-end, followed by neutralization with 0.5 ml of 1 M Tris-HCl pH 7.4. Alternatively, specific phage binders were recovered by elution with 1 ml of either 1 µM OPGbp[143-317] or 1 µM OPG[22-194]-Fc in 0.4% MPBS, pH 8.0 or pH 7.4, respectively.

Eluted phage (binders) were titered on E. coli strain TG1 (Pharmacia, Piscataway, NJ.). Titering was performed in duplicate by a modification of the "Track-Dilution" method (Huycke et al. BioTechniques 23, 648-650 (1997)) by 10 µl phage dilution in 2xTY broth into 90 µl log phase (A600 0.2 to 1.0 ODs) TG1 cells, mixed and incubated 20-30 minutes at room temperature. Ten



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$\mu$ l were streaked horizontally in a lane, 6 lanes per 2xTY-AG square petri dish (2xTY broth, containing 2% glucose, 100  $\mu$ l/ml ampicillin and 15 g/liter agar), and incubated overnight at 37°C.

5           The eluted phage (binders) were amplified through bacterial infection in TG1 cells. Twenty-five ml of 2xTY broth were inoculated with *E. coli* TG1 cells and grown at 30°C for more than 12 h, 270 rpm. The overnight culture was inoculated 1:100 in 50 ml of 2xTY  
10   broth, and grown ~1.5 hr, 270 rpm to an OD600 of 0.5. For amplification of selected phage, 5 volumes of exponential *E. coli* TG1 cells were added, 4 volumes of 2xTY broth and 1 volume of eluted (neutralized) phage together and incubated in a waterbath at 37 °C for 30  
15   min. To reduce the volume for plating the cells were centrifuged at 4,000 rpm and the pellet was resuspended in 2x TY-AG broth (100 ug/ml ampicillin, 2% glucose). In the first round of selection, the sample was plated onto two to four 16 cm<sup>2</sup> 2x TY-AG plates (2xTY broth,  
20   containing 2% glucose, 100 ug/ml ampicillin and 15 g agar) to maintain diversity. For later rounds of selection, one plate was sufficient. The plates were incubated overnight at 30 °C. After overnight growth, 5 mls of 2xTY-AG was added to each large plate, and  
25   bacteria were scraped loose with a sterile spreader. After complete resuspension and concentration by spinning down at 4,000 rpm, 10 min, a concentrated sample was transferred to a Nunc Cryotube. Sterile glycerol was added to 15% final concentration and  
30   immediately stored at -70 °C.

          Amplified cells were resuspended in 2x TY-AG broth to ~0.1 OD and grown for 1.5-2.5 h at 37°C, 270 rpm, to an OD600 of 0.5 and transferred (5 ml) to a 50-ml Falcon tube containing an appropriate amount of  
35   M13K07 helper phage (Gibco BRL Products, Grand Island, NY), with a 20 to 1 ratio of phage to bacteria. The

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mixture of phage and bacteria were incubated at 37°C for 30 min without agitation followed by centrifugation for 15 min, 3,700 rpm. The supernatant was removed and the bacterial pellet was resuspended in 25 ml of 2xTY-AK (100 ug/ml ampicillin, 25 ug/ml kanamycin) and transferred to a 250 ml flask for overnight incubation at 30°C with shaking at 270 rpm. Next day, the culture was centrifuged in a 50-ml Falcon tube for 20 min at 3,700 rpm to pellet the bacteria. To the supernatant, 1/5th of the volume of a polyethylene glycol (PEG) solution (20% PEG 8000, 2.5M NaCl) was added and kept on ice for at least 1 hr. Phage were pelleted 20 min, 3,700 rpm at 4°C. Supernatant was discarded and the pellet was resuspend in ~1.0 ml sterile PBS and transferred to a 1.5 ml eppendorf tube. The sample was microcentrifuged 2 min. ~14,000 rpm to remove the remaining bacteria and the supernatant was transferred to a new tube. The PEG precipitation was repeated. The concentrated PEG precipitated phage were used in selection or screening assays. The standard yield was about  $1-5 \times 10^{13}$  phage from a 25 ml culture. For longer storage, glycerol was added to the phage (15% final concentration) and the phage were stored at -70°C.

This procedure describes one round of screening, comprising the steps of binding, elution and amplification. Typically, three to five rounds of screening were performed in order to obtain an eluted phage pool which bound OPGbp [143-317] in an ELISA assay at a level at least four fold over background. After screening was completed, the final eluted phage were plated for individual colonies and the inserted DNA analyzed by colony PCR and BstNI digestion as described below.

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Solution phase

Phage were preblocked for 60 min on a rotator at room temperature in 2% MPBS. Biotinylated OPGbp b-b' loop peptide (500 nM) was added directly into the equilibrated phage mix and incubated for 30 min to 1 hour on a rotator (end-over-end) at room temperature. The loop peptide had the sequence [biotin(LC)-TDIPSGSHKVSLSWYHDRG] (SEQ ID NO: 24) where the LC (linear chain i.e.,  $(CH_2)_5-NH_2$ ) was used to link the OPGbp b-b' loop sequence to biotin. Streptavidin-coated Dynabeads (100  $\mu$ l per selection in 1.5 ml eppendorf tubes) were used for solution phase capture of biotinylated antigen-phage complexes (3X for negative and 1X for target antigen selection). Streptavidin-coated beads were pre-equilibrated by being drawn to the side of the tube using a Dynal magnet, buffer was removed and beads resuspended in 1 ml of 2% MPBS. Equilibration at room temperature was for 1-2 h on an end-over-end rotator.

Three negative selections were performed in rounds 2 and 3. For negative selection preblocked phage were added to a tube of streptavidin coated Dynabeads pre-equilibrated in 2% MPBS and incubated for 30 min on an end-over-end rotator at room temperature (repeated two times). Beads were drawn to the side and unbound phage transferred into a fresh eppendorf tube to be used for antigen selection. Biotinylated huOPGbp b-b' loop peptide (500 nM) was added directly into the equilibrated phage mix and incubated for 30 min to 1 hour on an end-over-end rotator at RT. Equilibrated beads were drawn to the side of the tube, buffer was removed and resuspended with the phage-biotinylated peptide mix followed by incubation for 15 min on an end-over-end rotator at RT. Tubes were placed in a magnetic rack for 1 min, aspirated and beads were washed 6x with 1 ml of 2% MPBS-Tween-20 (0.1%), 6x with

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1 ml of PBS-Tween-20 (0.1%), and 2x with 1 ml of PBS. Phage were eluted with 1 ml of 100 mM TEA pH 12 for 5-10 min on a rotator at room temperature followed by neutralization with 0.5 ml of 1M Tris-HCl, pH 7.4.

5

### Example 3

#### Identification of Fab clones which bind OPGbp

10           *E. coli* TG1 cells were infected with the phage pool from an ELISA responsive round and individual colonies were picked for PCR analysis. Typically one to four plates of 96 colonies were picked for each selection. Fab cDNAs were amplified by PCR by  
15 a specific set of primers and analyzed on an agarose gel for Fab insert length. Fab insert lengths > 1.6 kb were full length. cDNAs were also digested with BstNI restriction enzyme and the banding pattern analyzed by electrophoresis on agarose gels. Clones which  
20 exhibited identical size PCR full-length inserts and identical BstNI banding patterns in two or more isolates were candidates for further analysis. Using the above criteria, the following Fabs were identified.

Fab pattern "P" was identified after solution  
25 phase screening using three rounds of elution with triethylamine, pH 12, followed by solid phase screening as described above using one round of elution with 1  $\mu$ M OPGbp[143-317].

Fab pattern "S" was identified by solution  
30 phase screening using three rounds of elution with triethylamine, pH 12, followed by solid phase screening as described above using two rounds of elution with 1  $\mu$ M OPG[22-194]-Fc.

Fab pattern "AT" was identified by solid  
35 phase screening as described above using four rounds of elution with 1  $\mu$ M OPG[22-194]-Fc.

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Fab pattern "Y" was identified by solid phase screening as described above using three rounds of elution with OPGbp[143-317].

Phage were prepared from individual colonies exhibiting Fab AT, Y, P and S patterns by the following procedure. Plasmid preparations were made and transformed into TG1 cells. PCR analysis confirmed the transformation of a full length insert. The cells were grown in either a deep well block (0.5 ml volume) or as a 10 ml culture. Phage were rescued by a 20:1 ratio of M13K07 helper phage/cells infection, PEG precipitated 1 time (as in the solid phase direct plating protocol) and resuspended in ~ 200 ul from a 2-ml well sized deep well block or ~500 ul from the 10 ml culture in PBS.

Phage titers were in the range of  $10^{11}$ - $10^{14}$  phage/ml into the ELISA. Titrations based on volume using a maximum of 50  $\mu$ l/well additions were performed giving a typical range  $10^9$ - $10^{11}$  phage/well in an ELISA. Phage ELISA was performed as previously described. The ELISA uses anti-M13-HRP conjugate for detection of bound phage with ABTS, a colorimetric substrate at 405 nm. Anti-M13 HRP conjugate was specific for the major coat protein VIII on the phage. Values were from single point determinations.

Results of an ELISA of a representative clone from each of the major patterns "AT", "Y", "P" and "S" was shown in Figure 1. All four Fab clones demonstrated significant reactivity with OPGbp[143-317]. All clone members from patterns "AT" and "Y" (27 members from 672 clones and 9 members from 96 clones, respectively) were sequenced found identical within their patterns. Therefore any pattern member will be representative of the entire pattern for patterns "AT" and "Y".

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Patterns "Q" (3 members from 96 clones), "X" (3 members from 96 clones) and "AB" (2 members from 96 clones) were also ELISA positive to plated OPGbp[143-317] as determined by a representative clone (Figure 1). Since the ELISA signals were considerably lower than patterns "AT", "Y", "P", and "S", and represented by only two to three members in 96 clones, they were assumed to have Kds in the  $\mu$ M range and were not analyzed further. Pattern "X" was only ELISA positive when the concentration of Tween-20 in the washes was reduced from 0.1% to 0.01%.

#### Example 4

##### 15 Purification of soluble Fabs

Phage containing Fabs "AT", "Y", "P" and "S" were infected into E. coli HB2151 (Pharmacia, Piscataway, NJ) and expression of Fab fragments was induced by addition of IPTG to 1 mM generally for at least 5 h, except that for pattern Y the IPTG levels were reduced to 0.25 mM. After induction, the cells (750 ml) were harvested by centrifugation and Fabs were released from the periplasmic space by osmotic shock.

25 The total pellet was resuspended in 8 ml of ice cold TES (0.2 M Tris , 0.5 mM EDTA, 17.1 % sucrose, pH 8.0), transferred to a 50 ml tube and incubated for 5 to 10 min on ice with occasional gentle shaking. Meanwhile, the empty tubes were washed with 8.8 ml  
30 TES/H<sub>2</sub>O (1:3) to recover the remaining cell pellet and added to the other cells and incubated another 20 min on ice. Cells were centrifuged at 14,000 rpm for 3 min and supernatant transferred from the slightly sloppy cell pellet to another 50-ml tube. The  
35 supernatant was again centrifuged at 14,000 rpm for 10 min at 4°C to remove residual cell contamination. The



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supernatant was referred to as the TES-released periplasmic fraction. The bacterial pellet was resuspended in 10 ml TES plus 15 mM MgSO<sub>4</sub>, incubated on ice for 15 min and centrifuged twice as above. The  
5 supernatant was referred to as the Mg-released periplasmic fraction. Bovine serum albumin (BSA; RIA grade, Sigma) was added as a carrier and stabilizer to each periplasmic fraction to a final concentration of 1 mg/ml and dialyzed overnight at 4°C in 2 L with 1  
10 exchange of Talon column buffer (20 mM Tris-HCl/0.1 M NaCl, pH 8.5) plus protease inhibitors at the final concentrations, Pefabloc 0.05 mg/ml, leupeptin 50 nM, aprotinin 0.06 µg/ml and pepstatin A 0.9 µg/ml.

The Fab-containing periplasmic extracts (TES  
15 and Mg-released) were subjected separately to batch method binding 1 h rocking at 4°C with 0.8 ml to 1.5 ml (1/20<sup>th</sup> the extract volume) preequilibrated Talon resin (Clontech), then batch method washing in at least 2Xs 20 column volumes of column buffer. The Talon resin was  
20 column packed, washed with 10 column volumes of column buffer, and 2 column volumes of column buffer plus 50 mM imidazole to release nonspecifically bound proteins. Purified Fabs were eluted with 2 to 3 column volumes of 200 mM imidazole, 4% glycerol. Purified extracts were  
25 then concentrated/exchanged in a Centricon 10 (Amicon, Inc. Beverly, MA) into PBS, pH 7.4 to a final concentration of 0.5 to 5 mg/ml. Purity of soluble Fab "AT" was determined on a Novex (San Diego, CA) 10% Bis Tris NuPAGE Gel with NuPAGE MOPS SDS Running Buffer  
30 (Nonreducing) and 4X LDS Sample Buffer (pH 8.45). Purified Fab samples containing the LDS Sample Buffer were heated at 70°C for 10 min and loaded 40 µl/lane. The gel was run at 200 volts, 20 min, then reduced to 50 volts ~1.5 h, stained with Novex Colloidal Coomassie

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Blue Stain ~14 h, and destained. Soluble Fab "AT" was determined to have greater than 98% purity.

5

Example 5

## Activity of purified anti-OPGbp Fabs

The activity of purified soluble Fabs "AT" and "Y" was analyzed by the following assays.

10

OPGbp/ODAR interaction inhibition assay

The interaction of human OPGbp [143-317] and its receptor ODAR was measured in a fluorescence energy transfer (FRET) assay. A 1 hour preincubation with OPGbp was added for increased sensitivity. A soluble extracellular domain of human ODAR was constructed as a fusion polypeptide with an amino terminal FLAG tag and a carboxy terminal human Fc region. The Fc region is recognized and bound by a polyclonal goat anti-human Fc specific allophycocyanin (APC) which is detected by absorbance at 665 nm. OPGbp [143-317] was labeled with Eu<sup>2+</sup> which is detected by absorbance at 620 nm. Binding of OPGbp to ODAR in this assay triggers a fluorescent energy transfer and is highly sensitive for competition curves. A decrease in the A<sub>665</sub>/A<sub>620</sub> absorbance ratio indicates inhibition of OPGbp binding to ODAR.

A concentration dependent inhibition of OPGbp-Eu binding to sODAR-Fc was observed for duplicate preparations of soluble Fabs "AT" and "Y". Soluble Fab "AT" (preparation A) inhibited OPGbp binding to soluble ODAR-Fc fusion with an IC<sub>50</sub> of 550 nM. Soluble Fab "Y" inhibited with an IC<sub>50</sub> of 6  $\mu$ M. The Fab "AT" clone 1B5 was from the predominant 27 member pattern from seven 96-well plates (all with same amino acid sequence) obtained by elution from OPGbp target using a solution

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of 1  $\mu$ M OPG[22-194]-Fc for 90 min. The Fab "Y" clone 1B4 was from the predominant 9 member pattern from one 96-well plate (all with same amino acid sequence) from the optimized plated OPGbp screen using a 1  $\mu$ M OPGbp elution for 90min. A second purification of Fab "AT", clone 6F11, (designated preparation B) yielded a similar IC50 of 440nM for PBS and IC50 of 354nM for TBS. A second purification of Fab "Y" (preparation B) yielded a similar IC50 of 4.1  $\mu$ M for TBS. The positive control was OPGbp[143-317] in TBS or PBS with corresponding IC50s of 0.89 and 0.93 nM, respectively. Similar IC50's were obtained with the duplicate preparations "A" and "B". Results of preparation "B" of Fabs "AT" and "Y" in TBS are shown in figure 2.

#### Bone Marrow assay

Soluble Fabs "AT" "Y" and "P" were purified as in Example 4 followed by two sequential endotoxin removal steps using a Polymyxin affinity column (~1 ml; BioRad, Hercules, CA) in PBS at room temperature according to the manufacturers instructions except as follows. To increase the probability of removing endotoxin bound to the Fab, after sample addition, a 100  $\mu$ l to 150  $\mu$ l aliquot was recycled from the bottom to the top of the column every 5 min for 2 to 2.5 hours. Fab was eluted with 3 column volumes of PBS with 4% glycerol.

The samples were tested for endotoxin using E-Toxate (Limulus Amebocyte Lysate) detection system (Sigma, St. Louis, MO) according to manufacturer's instructions. The samples were then filter sterilized (0.2  $\mu$ m). Fab "P" was denatured after purification and bound poorly to OPGbp [143-317] in an ELISA. It was used as a negative control in these experiments.

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The assay format includes a 1 hour pre-incubation of the anti-OPGbp Fab with 10 ng/ml of human OPGbp [143-317] (final cell well concentration). TRAP assays were carried out in solution using pNPP chromogenic substrate. The results are shown in Figure 3. Fab "AT" gave a 50% decrease (IC<sub>50</sub>) at 57.8 nM; Fab "Y" gave a 50% decrease at 212 nM; Fab "P" gave a 50% decrease at 1.5  $\mu$ M. An estimated Fab molecular weight of 50,000 was used in these calculations to give a conversion factor of 1  $\mu$ g/ml = 20 nM.

The IC<sub>50</sub>'s as determined by TRAP histochemical staining were similar to those above as determined in the pNPP assay.

#### 15 RAW Cell Assay

The effects of adding soluble Fabs "AT", "Y" and "P" to the RAW cell assay are shown in Figure 4. The 50% point for the graph was taken as 1.65 OD<sub>405</sub> nm. Fab "AT" has an IC<sub>50</sub> of 15  $\mu$ g/ml, 300 nM (assuming a Fab molecular weight of 50,000). Fab "Y" has a decrease in signal to 2.15 OD<sub>405</sub> from 2.5 OD<sub>405</sub>, an 80% point. By extrapolation, a 50% point OD<sub>405</sub> of 1.65 for Fab "Y" would be reached at  $\sim 400 \mu$ g/ml  $\times 20 = \sim 8 \mu$ M. "P" does not show any detectable reactivity in the assay.

25

#### Example 6

##### Sequencing and Analysis of Fab clones

30 The DNA and predicted amino acid sequences for the light chains of Fabs "AT", "Y", "P" and "S" were shown in Figures 5, 6, 7, and 8 respectively. The DNA and predicted amino acid sequences for the heavy chains of Fabs "AT", "Y", "P" and "S" were shown in Figures 9, 10, 11, and 12, respectively. Figure 13 shows an amino acid sequence comparison matrix of the heavy and light

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chains respectively of the four predominant Fab pattern clones based on identity and similarity. Identity and similarity were obtained by either the GCG program or calculated by hand. The heavy chain sequences of Fabs "AT" and "Y" has the closest match as they differed by a single amino acid (conservative change) and thus had an identity of 99.6% and a similarity of 100%. The light chain amino acid sequences were compared among the top four patterns for both identity and similarity to each other. Fabs "AT", "Y" and "P" showed an identity of at least 85% and a similarity of at least 89%. Pattern "S" was the most dissimilar being of the rarer V lambda family.

A comparison of the amino acid sequences of the complementary determining regions (CDRs) was shown in Figure 14. Fabs "AT" and "Y" had identical heavy chain amino acid sequences in CDR1, CDR2 and CDR3. The heavy chain CDR1 of Fabs "P" and "S" each had 3 amino acid residues identical to the "AT" and "Y" CDR1 sequence. The CDR2 sequence of "P" and "S" showed greater identity to each other than to the "AT" and "Y" CDR2 sequence. The light chains of Fabs "AT" and "Y" were showed identical lengths of CDR1, CDR2 and CDR3. Patterns "AT" and "Y" light chain CDRs 1 and 3 showed identity in 7 of 11 residues (64%) and 3 of 5 residues (60%), respectively. Although patterns "AT" and "Y" light chain CDR2 show no identity to each other, each contain part of the consensus sequence for light chain CDR2. When the first 4 residues of pattern "AT" were combined with the last 3 residues of pattern "Y", the light chain CDR2 of pattern "P" was obtained. The light chain CDR3 can vary in length from 5 to 25 residues. Therefore, the light chain CDR3s obtained in patterns "AT", "Y" and "P" were very short. The most unique of the four predominant pattern clones was Fab "S."

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A comparison of the Fab classes represented in the four predominant patterns was shown in Figure 15. These results were obtained from V-Base DNA PLOT Analysis. As expected Fabs "AT" and "Y" were of the same VH1 family (Variable Heavy 1 family) with the same VDJ regions (Variable, Diversity and Joining). Fabs "AT" and "Y" belong to different light chain families. Fabs "P" and "S" belong to the same VH3 heavy chain family but different light chain families. All heavy chains have the same JH4b joining regions.

An alignment of the Fab sequences and the corresponding germ line sequences is shown in Figures 16-18 for the heavy chains and Figure 19-22 for the light chains. Changes in the heavy and light chains of "AT", "Y", "P" and "S" result from naturally occurring somatic mutations that arise in antibody germline sequences during an antibody response. Variable regions of the "AT" and "Y" heavy chains (the amino terminal 127 amino acids in Figures 9 and 10, respectively) had 17 and 18 amino acid changes, respectively, compared to the corresponding VDJ germline sequences. The variable region of the "P" heavy chain (the amino terminal 117 amino acids in Figure 11) has 16 amino acid changes compared to corresponding VDJ germline sequence. The variable region of the "S" heavy chain (the amino terminal 124 amino acids in Figure 12) had 14 amino acid changes compared to germline sequences. The variable region of the "AT" light chain (residues 6-108 in Figure 5) had 16 amino acid changes compared the corresponding VJ germline sequence; the "Y" light chain (residues 6-108 in Figure 6) had 14 amino acid changes; the "P" light chain (residues 5-108 in Figure 7) had 14 amino acid changes; and the "S" light chain (residues 5-112 in Figure 8) had 12 amino acid changes. In general, amino acid differences in the occurred with the most



10 Fab clones were converted to full-length antibodies by the following procedures.

The plasmid pDSR $\alpha$ 19:EPO was digested with HindIII and SalI to remove the coding region for erythropoietin. Plasmid pCRBluntCH1-3 containing the human IgG1 C<sub>H</sub>1, hinge, C<sub>H</sub>2 and C<sub>H</sub>3 domains inserted into the vector pCRBlunt (Invitrogen) was used to obtain a 1.4 kb human IgG1 constant domain. pCRBluntCH1-3 was digested with HindIII and Sal I and the constant domain sequences were inserted into pDSR $\alpha$ 19 to generate pDSR $\alpha$ 19:hCH.

Four anti-huOPGbp Fab heavy chain cDNAs were  
25 cloned into pDSR $\alpha$ 19:hCH to convert the Fabs into full  
length IgGs. The construction of a plasmid encoding  
"AT" heavy chain is described here. The other Fab  
heavy chains were cloned using similar procedures. To  
generate the Fab with a signal sequence, a three-step  
30 PCR was performed. First, primers 2249-25 and 2248-22  
were used with the Fab cDNA template. Conditions  
were: 94°C for 1 min, (95°C for 30 sec., 50°C for 1  
min., 68°C for 1 min) for 20 cycles, 68°C for 10 min.  
with Pfu polymerase and the appropriate buffer and

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nucleotides. The PCR product was then amplified with primers 2209-21 and 2248-22 followed by amplification with primers 2209-20 and 2248-22. The final PCR product was cleaned, cut with HindIII and BsmBI, and gel purified. This fragment contains the Fab with a 5' Kozak (translational initiation) site and the following signal sequence for mammalian expression of Fab "AT" heavy chain:

MEWSWVFLFFLSVTTGVHS (SEQ ID NO: 25)

10 This signal sequence is designated the VH2<sub>1</sub> signal sequence.

The plasmid pDSR $\alpha$ 19:hCH was digested with HindIII and BamBI to remove the polylinker before the IgG C<sub>H</sub>1-3 domains and the Fab PCR product was inserted to generate pDSR $\alpha$ 19:AT-VH21.

#### Primers:

20 HindIII Kozak  
2209-20 5'-CAGAAGCTTAGACCACC ATG GAA TGG AGC TGG GTC TTT CTC TTC T-3'  
(SEQ ID NOS: 26 & 27)  
M E W S W V F L F

25 2209-21 5'-G AGC TGG GTC TTT CTC TTC TTC CTG TCA GTA ACG ACT GGT GTC CA-3'  
(SEQ ID NOS: 28 & 29)  
S W V F L F F L S V T T G V

30 2249-25 5'-TCA GTA ACG ACT GGT GTC CAC TCA CAG GTC CAG CTG GTG CAG-3'  
(SEQ ID NOS: 30 & 31)  
S V T T G V H S Q V Q L V Q

BsmBI  
2248-22 5'-GTG GAG GCA CTA GAG ACG GTGACC AGG GTG-3' (SEQ ID NO: 32)

#### 35 Construction of pDSR $\alpha$ 19:AT-L

Fabs "AT", "Y", "P" and "S" light chain cDNAs were cloned into pDSR $\alpha$ 19 to convert the Fabs into full length antibodies. The construction of a plasmid encoding the "AT" light chain is described here. The other Fabs were cloned using similar procedures. To generate Fab "AT" with a signal sequence, a three-step PCR was performed. First, primers 2233-50 and 2233-51 were used with the Fab cDNA template. The PCR

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conditions were: 94°C for 1 min., (95°C for 30 sec., 50°C for 1 min., 68°C for 2 min.) for 20 cycles, 68°C for 10 min. with Pfu polymerase and the appropriate buffer and nucleotides. The PCR product was then amplified with primers 2148-98 and 2233-51 followed by amplification with primers 2148-97 and 2233-51. The final PCR product was cleaned, cut with HindIII and SalI, and gel purified. This fragment contains the Fab with a 5' Kozak (translational initiation) site and the following signal sequence for mammalian expression of the "AT" light chain:

MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO: 33)

This signal sequence is designated the "light" signal sequence.

The plasmid pDSRα19:EPO was digested with HindIII and SalI to remove the EPO gene before the IgG light chain PCR product was inserted to generate of pDSRα19:AT-L.

#### 20 Primers:

HindIII Kozak  
2148-97 5'-CAGAAGCTTGACCACC ATG GAC ATG AGG GTC CCC GCT CAG CTC CTG GG-  
3' (SEQ ID NO: 34 & 35)

25 M D M R V P A Q L L  
2148-98 5'-CC GCT CAG CTC CTG GGG CTC CTG CTA TTG TGG TTG AGA GGT GCC AGA  
T-3' (SEQ ID NO: 36 & 37) A Q L L G L L L L  
W L R G A R

30 2233-50 5'-G TGG TTG AGA GGT GCC AGA TGT GAA ATT GTG ATG ACA CAG TCT C-  
3' (SEQ ID NO: 38 & 39) W L R G A R C E I V M T Q S

SalI  
35 2233-51 5'-TTTGACGTCGAC TTA TTA ACA CTC TCC CCT G-3' (SEQ ID NO: 40 &  
41) \* \* C E G R

#### 40 Construction of pDSRα19:AT-tPA

Expression vectors for production of "AT", "Y", "P" and "S" full-length heavy chains were constructed as described above except that primers were modified to introduce the following signal sequence:

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MDAMKRGLCCVLLLLCGAVFVFSPSRGRFRR (SEQ ID NO:

42)

This signal sequence is designated the "tPA-RGR" signal sequence.

5

#### Construction of pLDH-AT

An expression vector was constructed that included both "AT" heavy and light chains. The plasmid pDSR $\alpha$ 19:AT-Vh21 was digested with AatII and NdeI and the ends filled by T4 DNA polymerase. The fragment containing the "AT" heavy chain expression cassette ("AT" heavy chain coding sequence flanked by the promoter and polyadenylation site from pDSR $\alpha$ 19) was gel purified and ligated to of pDSR $\alpha$ 19:AT-L which had been linearized with NheI, filled with T4 DNA polymerase and dephosphorylated with alkaline phosphatase. The heavy chain expression cassette was in the same transcriptional orientation as the light chain and DHFR genes.

20

#### Antibody Preparation

Expression vectors containing cDNA encoding heavy and light chain "AT", "Y", "S" and "P" full-length antibodies (either in separate vectors or in a single vector) were transfected into CHO cells and cultured under conditions to allow expression of heavy and light chains and secretion into the cell media. The conditioned media was filtered through a 0.45  $\mu$ m cellulose acetate filter (Corning, Acton, MA) and applied to a Protein G sepharose (Amersham Pharmacia Biotech, Piscataway, NJ) column which had been equilibrated with PBS - Dulbecco's Phosphate Buffered Saline without calcium chloride and without magnesium chloride (Gibco BRL Products, Grand Island, NY). After sample application the column was washed with PBS until

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absorbency at 280 nm reached baseline. Elution of protein was achieved using 100 mM Glycine, pH 2.5. Fractions were collected and immediately neutralized by addition of 1M Tris-HCl, pH 9.2. Antibodies were  
5 detected by SDS-polyacrylamide gels visualized by Commassie staining.

Fractions containing antibody were pooled, concentrated and diafiltered into PBS using either Centricon 10 (Amicon) or for larger volumes Centriprep  
10 10 (Amicon).

The isolated antibody was characterized by gel filtration on Superose 6 (Amersham Pharmacia Biotech, Piscataway, NJ) and was shown to run as a monomeric IgG.  
15

#### Example 8

#### BIACore Analysis of Fab and antibody binding to OPGbp

20 The binding constant ( $K_d$ ), on rate constant ( $k_a$ ) and off rate constant ( $k_d$ ) for Fabs "AT" and "Y" and "AT" antibody were determined by surface plasmon resonance techniques (BIACore, Pharmacia, Piscataway, New Jersey) and the results are shown in Table II.  
25 Fabs were prepared as described in Example 4 and "AT" antibody prepared as described in Example 7. OPGbp was immobilized on a CM5-chip by standard amine coupling. The  $K_d$  and rate constants were determined by BIAEVALUATION 3.0 software (Pharmacia).  
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TABLE II

BIAcore Analysis of Fabs "AT" and "Y" and "AT" antibody

	$K_a (M^{-1}s^{-1})$	$K_d (s^{-1})$	$K_D (nM)$
5 Fab "Y"	$9.44 \times 10^3$	$5.91 \times 10^{-3}$	594
Fab "AT"	$2.2 \times 10^6$	0.31	140
"AT" Ab (147 RU)	$5.7 \times 10^6$	$2.4 \times 10^{-3}$	0.42
"AT" Ab (80 RU)	$8.1 \times 10^6$	$2.7 \times 10^{-3}$	0.33
10 "AT" Ab (47 RU)	$7.3 \times 10^6$	$3.1 \times 10^{-3}$	0.43

The binding affinity of Fab "AT" increased from about 140 nM to about 0.33 to 0.43 nM, or about 350 to 400-fold, when an Fc IgG<sub>1</sub> constant region was added as described in Example 7.

Example 9

## Activity of anti-OPGbp antibodies

20

RAW Cell Assay

"AT" antibody preparations designated 405, 406 and 407 were tested in a RAW cell assay as described in Example 1. "AT"405, "AT"406 and "AT"407 differ only in the leader sequences used for expression ("AT"405 was expressed using the "light" signal sequence, "AT"406 used the tPA-RGR signal sequence, and "AT"407 used the VH21 signal sequence). The purified mature antibodies were identical in each preparation. The results are shown in Figure 23.

IC50's for "AT"405, "AT"406 and "AT"407 were 20.1 nM, 60.3 nM & 21.4 nM, respectively corresponding to 3.0 ug/ml, 9.0 ug/ml and 3.2 ug/ml, respectively (assuming a molecular weight of 150,000). The positive controls of OPG[22-194]-Fc and anti-OPGbp



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polyclonal antibody were at 33 ng/ml and 150 ng/ml, respectively.

The difference in IC50 in the Raw cell assay of the "AT" Fab fragment was 300 nM as compared to about 20 nM for the "AT" full-length, or about a 15-fold increase for the "AT" full-length antibody. Taking into account the differences in OPGbp concentrations in the two experiments (150 ng/ml / 60 ng/ml = 2.5 fold), the increase in cell function avidity for the "AT" full-length antibody was about 15 x 2.5 = 37.5 fold.

#### Bone Marrow Assay

The effects of adding "AT"405 or "AT"407 preparations to the murine bone marrow assay were shown in Figure 24. IC50's for "AT"405 and "AT"407 were 2.15 ug/ml (14.4 nM), and 1.85 ug/ml (12.5 nM), respectively, (MW = 150,000). A rat anti-OPGbp polyclonal antibody inhibited TRAP formation with an IC50 at ~50 ng/ml.

In a separate experiment, "AT"406 was tested in the bone marrow coculture assay and inhibited TRAP formation with an IC50 of 2.35 ug/ml (14.4 nM) assuming an antibody molecular weight of 150,000 (see Figure 25).

The difference in IC50 in the bone marrow assay for "AT" Fab fragment was about 50 nM as compared to about 13 nM for "AT" full-length antibody, or about a 3.85 fold increase. Taking into account the differences in OPGbp concentrations in the two experiments (50 nM / 13 nM = 3.85 fold), the approximate gain in cell function avidity from "AT" full-length antibody was about 3.85 x 3.85 = 14.8 fold.

cDNAs encoding Fabs "Y", "P" and "S" were also fused to human IgG1 CH1, CH2, and CH3 sequences as described in Example 7 and transfected into CHO cells.

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The resulting antibodies were expressed and purified from conditioned medium and endotoxin removed as before. "S" and "Y" light chain antibodies (referred to as "S Light" and "Y Light") were tested in the bone marrow assay and the results shown in Figure 26. "S light" and "Y light" antibodies were expressed using the corresponding light chain leader sequences and were designated "S" 435 and "Y" 429, respectively. The "Y" 429 ("Y Light") had an IC50 of 23 ug/ml or 154 nM. "S" 435 ("S Light") did not exhibit sufficient activity for a determination of an IC50.

The "Y Campath" and "P Light" were Hu-IgG1 sequence "Y" and "P", respectively with the leader sequence from the "Campath" and "Light" Chain, and were designated "Y" 442 and "P" 444, respectively. The "Y" 442 ("Y Campath") had an IC50 of 20 ug/ml or 134 nM. "P" 444 ("P Light") did not show detectable activity (see Figure 27).

The difference in IC50 in the bone marrow assay of "Y" Fab fragment was 212 nM compared to 134-154 nM for "Y" full-length antibody or about a 1.38 to 1.58 fold increase. Taking into account the differences in OPGbp concentrations in the two experiments (50 nM / 9 nM = 5.55 fold), the approximate gain in cell function avidity from "Y" Fab to "Y" full-length antibody was therefore about (1.38 to 1.58) x 5.55 = 7.7 to 8.8 fold or about 8-fold.

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#### Example 10

Identification of an Epitope for  
"AT" Antibody on OPGbp

#### Production of variant murine OPGbp

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Human OPGbp[143-317] was produced as described in Example 1. Murine OPGbp[158-316]

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containing amino acid residues 158 through 316 of as shown in Figure 1 of PCT W098/46751 preceded by an introduced N-terminal methionine residue was produced in *E.coli* and purified from the soluble fraction of bacteria as described previously (Lacey et al. Cell 93, 165-176 (1998)). FLAG-murine OPGbp[158-316] was generated by introduction of nucleic acid residues encoding an N-terminal methionine followed by a FLAG-tag sequence (DYKDDDDKKL (SEQ ID NO: 99)) fused to the N-terminus of residues 158-316 as shown in Figure 1 of PCT W098/46751 using methods known to one skilled in the art. The FLAG-OPGbp[158-316] molecule was cloned into bacterial expression vector pAMG21 (deposited with the American Type Culture Collection and having accession no. 98113).

A FLAG-murine OPGbp[158-316] polypeptide variant was constructed in which amino acid residues SVPTD at positions 229-233 inclusive as shown in Figure 1 (SEQ ID NO: 1) of W098/46751 were substituted with corresponding amino acid residues DLATE at positions 230-234 inclusive as shown in Figure 4 (SEQ ID NO: 3) of W098/46751. The resulting construct referred to as "FLAG-murine OPGbp[158-316]/DE" has the nucleic acid and protein sequence as shown in Figure 28. The amino acid sequence changes are located in a region of OPGbp between the D and E regions. Figure 29 shows a comparison of murine, human, and murine DE variant amino acid sequences in this region. The sequence changes in the murine variant are S229D, V230L, P231A and D233E with the T at position 234 unchanged. Flanking sequences in this region are virtually identical between murine and human OPGbp.

This molecule was constructed using a two step PCR reaction where the first step contained two separate PCR reactions, designated reaction A and reaction B. For both reaction A and reaction B,

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pAMG21-FLAG-murine OPGbp[158-316] DNA was used as a template for PCR. Reaction A employed oligonucleotides #2640-90 and #2640-91 for PCR, whereas reaction B employed oligonucleotides #2640-92 and 2640-93.

- 5 Thermocycling was performed and PCR products from reactions A and B were purified from an agarose gel using methods available to one skilled in the art. The second step PCR reaction, designated reaction C, utilized purified reaction A and reaction B PCR
- 10 products as a template and oligonucleotides #2640-90 and #2640-93 as primers. Following thermocycling, the product from reaction C was cloned into the pCRII-TOPO cloning vector (Invitrogen) & electroporated into DH10b (Gibco) cells using methods provided by the
- 15 manufacturer. Clones were selected and sequence confirmed verifying that the introduced mutations resulted in changing the amino acid sequence SVPTD in murine OPGbp[158-316] to DLATE. The sequence verified DNA was then digested with NdeI and XhoI, purified, and
- 20 subcloned into bacterial expression vector pAMG21 giving rise to plasmid pAMG21-FLAG-murine OPGbp[158-316]/DE.

2640-90: CCTCTCATATGGACTACAAGGAC (SEQ ID NO: 100)

- 25 2640-91: AGTAGCCAGGTCTCCCGATGTTTCATGATG (SEQ ID NO: 101)

2640-92: CTGGCTACTGAATATCTTCAGCTGATGGTG (SEQ ID NO: 102)

2640-93: CCTCTCCTCGAGTTAGTCTATGTCC (SEQ ID NO: 103)

30

- E.coli host GM94 (deposited with the American Type Culture Collection under accession number 202173) containing plasmid pAMG21-FLAG-murine OPGbp[158-316]/DE was grown in 2XYT media to an exponential growth phase
- 35 and induced to express the FLAG-murine OPGbp[158-316]/DE protein by addition of *V. fischeri* synthetic

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autoinducer to 100 ng/ml. Approximately 3-6 hours following induction, the cells were pelleted and recombinant FLAG-murine OPGbp[158-316]/DE protein was purified from the soluble fraction of *E.coli* using methods described in Lacey et al. ibid.

10 Binding of "AT" Antibody to human OPGbp[143-317], murine OPGbp[158-316], and FLAG-murine OPGbp[158-316]/DE

Costar E.I.A./R.I.A. Plates (Flat Bottom High Binding, Cat.#3590) were coated with 100 µl/well of either human OPGbp[143-317]protein, murine OPGbp[158-316] protein, or FLAG-murine OPGbp[158-316]/DE protein at 3 µg/ml in PBS, overnight at 4°C with agitation. After overnight incubation, the protein solutions were removed from the plate and 200 µl of 5% Chicken Serum (Gibco/BRL Cat# 16110-082) in PBST (PBS plus 0.05% Tween 20) was added to each well of the plate and plates were incubated at room temperature (RT) for 90 min with agitation. After incubation and blocking, plates were washed 4 times with 1X K-P wash solution in dH<sub>2</sub>O (Cat#50-63-00, Kirkegaard & Perry Laboratories) and dried. Purified "AT" antibody or human OPG [22-194]-Fc protein was serially diluted 1:1 from 2 ug/ml down to 1.953 ng/ml in PBST and 100 ul/well was added to appropriate wells of the microtiter plate coated with either human OPGbp[143-317], murine OPGbp[158-316], or FLAG-murine OPGbp[158-316]/DE protein. Plates were incubated for three hours at room temperature with agitation, washed four times with 1X K-P wash solution and dried. Goat anti-human IgG (Fc) (Jackson ImmunoResearch, Cat# 109-036-098) was diluted 1:5000 in 5% Chicken Serum in PBST and 100 µl was added to each well. Plates were incubated for 1.25 hours at

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room temperature with agitation, washed six times with 1X K-P wash solution, and dried. 100  $\mu$ l of undiluted ABTS substrate (Kirkegaard & Perry; Cat# 50-66-00) was added to each well and the dish was incubated at room temperature until sufficient blue-green color developed. Color development was stopped by addition of 100  $\mu$ l 1% SDS. Quantitation of color development was performed using a microtiter plate reader with detection at 405 nm.

10           The results of the EIA are shown in Figure 30. The "AT" antibody binds to human OPGbp[143-317] but does not show detectable binding to murine OPGbp[158-316] over the antibody concentration range tested. However, the "AT" antibody binds to both FLAG-murine OPGbp[158-316]/DE and to human OPGbp[143-317] under the assay conditions above. It was concluded that the amino acid changes in murine OPGbp[158-316]/DE compared to murine OPGbp[158-316] were involved in binding of "AT" antibody.

20           The FLAG-murine OPGbp[158-316]/DE was assayed for activity in a RAW cell assay as described in Example 1 and observed to have a similar ED50 for osteoclast formation as human OPGbp[143-317], indicating that the DE variant is active in promoting osteoclast activity in vitro. Therefore, the binding of "AT" antibody to murine OPGbp[158-316]/DE is likely to inhibit osteoclast formation.

          The epitope of the "AT" antibody is located to a region of human OPGbp which includes at least amino acids residues DLATE (residues 230 through 234 of human OPGbp as shown in Figure 4 of PCT W098/46751).

#### Example 11

35           Construction of "AT" Fab and Antibody Variants



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Three approaches were used to generate CDR variants of "AT" antibody: alanine scanning mutagenesis of heavy chain CDR3 region, substitution mutagenesis of selected sites in heavy chain CDR3 region, and insertion mutagenesis of the light chain CDR3 region.

Alanine variants of heavy chain CDR3

Alanine scanning mutagenesis was done on the CDR3 region of the AT heavy chain. The amino acid sequence used for alanine scanning was:

DSSNMVRGIIIIAYYFDY (SEQ ID NO: 104)

Primers 12 and 15 were annealed to each other and extended by polymerase chain reaction (PCR) under the following conditions: 25 pmol of each primer, 6 cycles of 30 sec at 94°, 2 min at 55°, 20 sec at 74°.

20

12) 5' AGA GAT TCC TCA AAT ATG GTT CGG GGA ATT ATT ATA GCG (SEQ ID NO: 105)

15) 5' GTA GTC AAA ATA GTA CGC TAT AAT AAT TCC CCG AAC (SEQ ID NO: 106)

25

The PCR product of this reaction is termed Template A.

Template A was extended by PCR using primers 11 and 14 under the following conditions: 0.5 microliters of template A, 10 pmol each primer, 15 cycles of 30 sec at 94°, 2 min at 43°, 20 sec at 74°.

30

11) 5' GTG TAT TAC TGT GCG AGA GAT TCC TCA AAT ATG (SEQ ID NO: 107)

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14) 5' CAG GGT GCC CTG GCC CCA GTA GTC AAA ATA GTA CGC  
(SEQ ID NO: 108)

The PCR product of this reaction is termed Template B.

5 Alanine variants of CDR3 were then generated  
using either template A or B. Two rounds of PCR were  
done (20 cycles of 94° C for 20 sec and 74°C for 40  
sec) using a primer containing the desired alanine  
codon. The primer containing the alanine codon was in  
10 the forward orientation for CDR3 residues (numbering  
follows Kabat system, see Kabat et al. Sequences of  
Proteins of Immunological Interest, U.S. Department of  
Health and Human Services, 4<sup>th</sup> edition (1991)) D95, S96,  
V100, R100a. The primer containing the alanine codon  
15 was in the reverse orientation for residues S97, N98,  
M99, G100b, I100c, I100d, I100e, Y100g, Y100h, F100i,  
D101, Y102.

The mutagenesis reactions were then followed by up to  
three extension reactions using six common primers.  
20 These reactions extended the product to encompass the  
entire CDR3 region up to and including unique flanking  
restriction sites BglII and BstEII.

Primers for alanine substitution are shown  
below in the 5' to 3' orientation. The alanine codon  
25 is shown in bold type.

60) 5' GTG TAT TAC TGT GCG AGA **GCT** TCC TCA AAT ATG GTT  
CGG (SEQ ID NO: 109)  
59) 5' GTG TAT TAC TGT GCG AGA GAT **GCC** TCA AAT ATG GTT  
30 CGG (SEQ ID NO: 110)  
58) 5' AAT AAT TCC CCG AAC CAT ATT **TGC** GGA ATC TCT CGC  
ACA GTA (SEQ ID NO: 111)  
57) 5' AAT AAT TCC CCG AAC CAT **AGC** TGA GGA ATC TCT CGC  
ACA (SEQ ID NO: 112)

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- 56) 5' AAT AAT TCC CCG AAC **GGC** ATT TGA GGA ATC TCT CGC  
(SEQ ID NO: 113)
- 55) 5' GAT TCC TCA AAT ATG **GCT** CGG GGA ATT ATT ATA GCG  
(SEQ ID NO: 114)
- 5 54) 5' GAT TCC TCA AAT ATG GTT **GCC** GGA ATT ATT ATA GCG  
TA (SEQ ID NO: 115)
- 53) 5' GTA GTC AAA ATA GTA CGC TAT AAT AAT **TGC** CCG AAC  
CAT ATT TGA (SEQ ID NO: 116)
- 52) 5' GTA GTC AAA ATA GTA CGC TAT AAT **GGC** TCC CCG AAC  
10 CAT ATT (SEQ ID NO: 117)
- 51) 5' GTA GTC AAA ATA GTA CGC TAT **GGC** AAT TCC CCG AAC  
CAT (SEQ ID NO: 118)
- 50) 5' GTA GTC AAA ATA GTA CGC **TGC** AAT AAT TCC CCG AAC  
(SEQ ID NO: 119)
- 15 20) 5' GGT GCC CTG GCC CCA GTA GTC AAA ATA **GGC** CGC TAT  
AAT AAT TCC (SEQ ID NO: 120)
- 19) 5' GGT GCC CTG GCC CCA GTA GTC AAA **AGC** GTA CGC TAT  
AAT AAT TCC (SEQ ID NO: 121)
- 18) 5' CAG GGT GCC CTG GCC CCA GTA GTC **AGC** ATA GTA CGC  
20 TAT AAT (SEQ ID NO: 122)
- 17) 5' CAG GGT GCC CTG GCC CCA GTA **GGC** AAA ATA GTA CGC  
TAT (SEQ ID NO: 123)
- 16a) 5' CAG GGT GCC CTG GCC CCA **GGC** GTC AAA ATA GTA CGC  
TAT (SEQ ID NO: 124)

25.

The six common primers for extension PCR are shown below. The sequences of the flanking BglII (top strand) and BstEII (bottom strand) sites are shown in bold type.

30

Forward Common primers

- 10) 5' AGT CTG **AGA** **TCT** GAA GAC ACG GCT GTG TAT TAC TGT  
GCG AGA (SEQ ID NO: 125)

35

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11) 5' GTG TAT TAC TGT GCG AGA GAT TCC TCA AAT ATG (SEQ  
ID NO: 126)

12) 5' AGA GAT TCC TCA AAT ATG GTT CGG GGA ATT ATT ATA  
5 GCG (SEQ ID NO: 127)

Reverse Common primers

13) 5' CTT GAG ACG GTG ACC AGG GTG CCC TGG CCC CA (SEQ  
10 ID NO: 128)

14) 5' CAG GGT GCC CTG GCC CCA GTA GTC AAA ATA GTA CGC  
(SEQ ID NO: 129)

15 15) 5' GTA GTC AAA ATA GTA CGC TAT AAT AAT TCC CCG AAC  
(SEQ ID NO: 130)

The following table shows the starting  
template and primer pairs used sequentially to build  
20 synthetic DNA fragments containing substituted alanine  
residues.

TABLE III

25

		PCR reactions			
CDR Residue	Template	Alanine Primer pair	Extension Primers	Extension Primers	Extension Primers
D95	A	60 + 14	10 + 13		
S96	A	59 + 14	10 + 13		
S97	B	10 + 58	10 + 15	10 + 14	10 + 13
N98	B	10 + 57	10 + 15	10 + 14	10 + 13
M99	B	10 + 56	10 + 15	10 + 14	10 + 13
V100	A	55 + 14	10 + 14	10 + 13	

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R100a	A	54 + 14	10 + 14	10 + 13	
G100b	A	11 + 53	10 + 14	10 + 13	
I100c	A	11 + 52	10 + 14	10 + 13	
I100d	A	11 + 51	10 + 14	10 + 13	
I100e	A	11 + 50	10 + 14	10 + 13	
Y100g	B	11 + 20	10 + 13		
Y100h	B	11 + 19	10 + 13		
F100i	B	11 + 18	10 + 13		
D101	B	11 + 17	10 + 13		
Y102	B	11 + 16a	10 + 13		

For primers used for alanine scanning, the PCR conditions were 20 cycles at 94° C for 20 sec, then 74°C for 40 sec. For extension reactions with primer pair 10 + 15, the conditions were 20-25 cycles of 94° C for 20 sec, 42° for 1 min 30 sec, 74°C for 20 sec. For extension reactions with primer pair 10 + 14, the conditions were 20-25 cycles of 94° C for 20 sec, 48-50° for 1 min 30 sec, 74°C for 20 sec. For extension reactions with primer pair 10 + 13, the conditions were 20-25 cycles of 94° C for 20 sec, 64° for 1 min 30 sec, 74°C for 20 sec. The PCR products were digested with BglII and BstEII restriction enzymes and cloned into FabAT which had been digested with BglII and BstEII, thereby replacing the CDR3 of AT with the alanine substituted CDR3. The alanine substituted constructs were verified by DNA sequencing.

20

#### Substitution variants of heavy chain CDR3

A similar strategy to that used for the alanine scanning mutagenesis was utilized for the

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randomization of positions S96, S97 and N98 of the heavy chain CDR3 of "AT". Templates A and B were generated as previously described. Positions 96, 97 and 98 were randomized with a set of four primer for each position. The positions in parentheses have variable nucleotides as indicated.

For position S96.

10 23) 5' AAT AAT TCC CCG AAC CAT ATT TGA G(AT)(ACGT) ATC  
TCT CGC ACA GTA (SEQ ID NO: 131)  
24) 5' AAT AAT TCC CCG AAC CAT ATT TGA G(CG)(ACGT) ATC  
TCT CGC ACA GTA (SEQ ID NO: 132)  
25) 5' AAT AAT TCC CCG AAC CAT ATT TGA CT(CGT) ATC TCT  
15 CGC ACA GTA (SEQ ID NO: 133)  
26) 5' AAT AAT TCC CCG AAC CAT ATT TGA C(AC)(AT) ATC  
TCT CGC ACA GTA (SEQ ID NO: 134)

For position S97

20  
27) 5' AAT AAT TCC CCG AAC CAT ATT G(AT)(ACGT) GGA ATC  
TCT CGC ACA GTA (SEQ ID NO: 135)  
28) 5' AAT AAT TCC CCG AAC CAT ATT G(CG)(ACGT) GGA ATC  
TCT CGC ACA GTA (SEQ ID NO: 136)  
25 29) 5' AAT AAT TCC CCG AAC CAT ATT CT(CGT) GGA ATC TCT  
CGC ACA GTA (SEQ ID NO: 137)  
30) 5' AAT AAT TCC CCG AAC CAT ATT C(AC)(AT) GGA ATC  
TCT CGC ACA GTA (SEQ ID NO: 138)

30 For position N98

31) 5' AAT AAT TCC CCG AAC CAT G(AT)(ACGT) TGA GGA ATC  
TCT CGC ACA (SEQ ID NO: 139)  
32) 5' AAT AAT TCC CCG AAC CAT G(CG)(ACGT) TGA GGA ATC  
35 TCT CGC ACA (SEQ ID NO: 140)



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33) 5' AAT AAT TCC CCG AAC CAT CT(CGT) TGA GGA ATC TCT  
CGC ACA (SEQ ID NO: 141)

34) 5' AAT AAT TCC CCG AAC CAT C(AC)(AT) TGA GGA ATC  
TCT CGC ACA (SEQ ID NO: 142)

5

PCR reactions were done using the reverse randomization primer and primer 10. The resulting product was extended by four sequential PCR reactions using primer pairs 10 + 15, 10 + 14, 10 + 13 and 10 + 22. The 5' end of the heavy chain variable region of "AT" was amplified from a full length clone of the "AT" heavy chain variable region using primers 16 and 72. All PCR products were gel purified. The randomization products were overlapped with the 16/72 product, with flanking primers being 16 and 22. The full-length variable region was then cloned into a vector containing the IgG1 constant region as a HindIII/BsmBI fragment. Full-length antibody clones were selected by sequencing.

20

Primers used the overlap PCR for randomization mutants.

10 5' AGT CTG AGA TCT GAA GAC ACG GCT GTG TAT TAC TGT  
25 GCG AGA (SEQ ID NO: 143)

16 5' CAG CAG AAG CTT AGA CCA CCA TGG ACA TGA GGG TCC  
CCG CTC AGC TCC TGG G (SEQ ID NO: 144)

30 72 5' CAC AGC CGT GTC TTC AGA TCT CAG ACT GCG CAG CTC  
(SEQ ID NO: 145)

22 5' GTG GAG GCA CTA GAG ACG GTG ACC AGG GTG (SEQ ID  
NO: 146)

35

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The S96A, S97A, and N98A variants were converted from Fabs to full length antibodies by PCR amplification of the Fab clone having the bacterial signal sequence replaced with a mammalian signal sequence. Plasmid DNA from the Fab containing the  
5 desired mutation was used as template. Sequential primer pairs used were 21 + 22, 98 + 22, and 16 + 22. Correct clones were selected by DNA sequence analysis.

Multiple alanine variants (S96A, S97A; S96A, N98A; S97A, N98A and S96A, S97A and N98A) were  
10 generated using the converted AT heavy chain full length IgG1 plasmid as template. Initial PCR reaction were carried out with one of the primers listed below and primer 22. These products were then extended using  
15 primer pairs 10 + 22. That product was then overlapped with the 16/72 product using flanking primers 16 and 22, and cloned into the IgG1 constant region as before. Correct clones were selected by DNA sequence analysis.

20 S96A, S97A

63) 5' GTG TAT TAC TGT GCG AGA GAT GCC GCA AAT ATG GTT  
CGG GGA ATT ATT (SEQ ID NO: 147)

25 S96A, N98A

64) 5' GTG TAT TAC TGT GCG AGA GAT GCC TCA GCT ATG GTT  
CGG GGA ATT ATT ATA GC (SEQ ID NO: 148)

30 S97A, N98A

65) 5' GTG TAT TAC TGT GCG AGA GAT TCC GCA GCT ATG GTT  
CGG GGA ATT ATT ATA GC (SEQ ID NO: 149)

35 S96A, S97A, N98A

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66) 5' GTG TAT TAC TGT GCG AGA GAT GCC GCA GCT ATG GTT  
CGG GGA ATT ATT ATA GC (SEQ ID NO: 150)

21) 5' GTG GTT GAG AGG TGC CAG ATG TCA GGT CCA GCT GGT  
5 GCA G (SEQ ID NO: 151)

98) 5' CCG CTC AGC TCC TGG GGC TCC TGC TAT TGT GGT TGA  
GAG GTG CCA GAT (SEQ ID NO: 152)

10

Insertion Variants of light chain CDR3 region

The AT light chain CDR3 contains a five amino acid loop having the sequence QHTRA (SEQ ID NO: 09). Light chain CDR3 sequence variants were constructed as follows. The following primers were used for PCR using  
15 a plasmid containing Fab "AT" light chain cDNA as a template:

(HincII)CCG GTC AAC ACA CT(ACGT) (ACGT) (GT)G  
CGG CGG CGC GGG CGT TCG GCC AAG GG (SEQ ID NO: 153)  
20 where (ACGT) indicates a mixed four bases in this position.

CCG GGC GCG CCT TAT TAA CAC TC(AscI) (SEQ ID NO: 154)

25 The resulting PCR product had a CDR3 loop sequence increased from five to nine amino acids and changed from QHTRA to GHTXAAARA where X can be any amino acid. The AT clone was digested with HincII and AscI digestion and the "AT" light chain CDR3 region was  
30 replaced with the variant sequence. Clones containing all twenty amino acid residues in the X position along with three alanine residues inserted in the CDR3 loop were isolated and their identity confirmed by DNA sequencing.

35

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Purification of Fab Variants

Alanine substitution variants of heavy chain CDR3 and insertion variants of light chain CDR3 were produced and purified as Fab fragments by the following procedure. Each variant was grown in 50 ml of 2XYT with 2% glucose and 100 $\mu$ g/ml ampicillin while shaking at 37°C up to an OD<sub>600</sub> of 0.8-1.0. Each culture was then spun down and resuspended in 50ml of 2XYT with 100 $\mu$ g/ml Ampicillin with 1mM IPTG at 30°C to induce production of soluble Fabs. The soluble Fabs were then migrated to periplasmic area and concentrated over-night prior to release by osmotic shock. Osmotic shock was carried on by washing the cells by cold 0.5M sucrose solution in Tris buffer and EDTA to break the bacterial cell wall and then quickly diluting it into cold solution of low osmotic strength. The released soluble Fabs were purified on TALON metal affinity chromatography via 6X His-tagged residues on the expressed Fab. The impurities was washed away with NaCl and lower Imidazole concentrations prior to eluting the protein by Imidazole. Expression and purification of each mutant was analyzed by reducing, non-reducing and anti-His western blots. Total protein concentration was determined by A<sub>280</sub>.

## Example 12

## BIAcore Analysis of "AT" variants

The binding constant (K<sub>d</sub>) on rate constant (K<sub>a</sub>) and off rate constant (k<sub>d</sub>) for "AT" antibody variants, and off rate constant (K<sub>a</sub>) for "AT" Fab variants were determined by surface plasmon resonance techniques (BIAcore) using immobilized OPGbp [143-317]. The results are shown in Tables IV - VIII. The light

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chain CDR3 variants described in Example 11 did not demonstrate detectable binding.

TABLE IV

BIACore Analysis of "AT" Fabs from  
Alanine Scanning Mutagenesis of Heavy Chain CDR3

	Kd(s <sup>-1</sup> )
AT (No substituted alanines)	0.284
D95A	No detectable binding
S96A	7.20x10 <sup>-3</sup>
S97A	6.20x10 <sup>-3</sup>
N98A	1.20x10 <sup>-2</sup>
M99A	1.12
V100A	0.261
R(100a)A	0.303
G(100b)A	0.657
I(100c)A	0.53
I(100d)A	0.622
I(100e)A	6.90x10 <sup>-3</sup>
A(100f)	0.197
Y(100g)A	7.40x10 <sup>-2</sup>
Y(100h)A	0.368
F(100i)A	0.251
D110A	0.127
Y111A	0.414

TABLE V

BIACore Analysis of "AT" Abs from  
Alanine Scanning Mutagenesis of Heavy Chain CDR3

	$K_a$ ( $M^{-1}s^{-1}$ )	$K_d$ ( $s^{-1}$ )	$K_D$ (nM)
AT	$1.70 \times 10^6$	$2.00 \times 10^{-3}$	1.18
S96A	$2.60 \times 10^5$	$6.40 \times 10^{-4}$	2.50
S97A	$5.30 \times 10^5$	$1.50 \times 10^{-3}$	2.90
N98A	$1.30 \times 10^6$	$9.00 \times 10^{-4}$	0.67
S96A, S97A	$3.17 \times 10^5$	$2.84 \times 10^{-3}$	8.90
S97A, N98A			No detectable binding
S96A, S97A, N98A			No detectable binding
S96A, N98A			No detectable binding

5

TABLE VI

BIACore Analysis of "AT" Abs  
Substituted at S96 of Heavy Chain CDR3

	$K_a$ ( $M^{-1}s^{-1}$ )	$K_d$ ( $s^{-1}$ )	$K_D$ (nM)
S96F	$3.17 \times 10^5$	$2.68 \times 10^{-3}$	8.45
S96Q	$1.16 \times 10^6$	$3.59 \times 10^{-3}$	3.09
S96M	$3.57 \times 10^6$	$8.70 \times 10^{-3}$	2.43
S96V	$5.37 \times 10^6$	$1.21 \times 10^{-2}$	2.25
S96I	$2.99 \times 10^6$	$4.66 \times 10^{-3}$	1.56
S96N	$1.40 \times 10^6$	$2.15 \times 10^{-3}$	1.53
S96P	$1.45 \times 10^6$	$1.88 \times 10^{-3}$	1.29
S96W	$1.61 \times 10^6$	$2.03 \times 10^{-3}$	1.26
S96T	$3.14 \times 10^6$	$1.67 \times 10^{-3}$	0.53
S96D	$2.28 \times 10^6$	$1.18 \times 10^{-3}$	0.51
S96R	$6.97 \times 10^6$	$2.62 \times 10^{-3}$	0.38
S96E	$2.54 \times 10^6$	$9.01 \times 10^{-4}$	0.35
S96K	$4.55 \times 10^6$	$1.41 \times 10^{-3}$	0.31



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S96L	$5.84 \times 10^6$	$1.75 \times 10^{-3}$	0.30
S96H	$4.12 \times 10^6$	$7.64 \times 10^{-3}$	1.86
S96G	$3.87 \times 10^6$	$1.50 \times 10^{-2}$	3.85
S96Y	$2.75 \times 10^6$	$1.85 \times 10^{-3}$	0.67

TABLE VII

5

BIACore Analysis of "AT" Abs  
Substituted at S97 of Heavy Chain CDR3

	$K_a$ ( $M^{-1}s^{-1}$ )	$K_d$ ( $s^{-1}$ )	$K_D$ (nM)
S97F			No detectable binding
S97Y			No detectable binding
S97G			No detectable binding
S97W			No detectable binding
S97D			No detectable binding
S97E	Fast on	Fast off	1940
S97R	$4.09 \times 10^6$	$1.18 \times 10^{-1}$	28.90
S97P	$1.10 \times 10^6$	$2.69 \times 10^{-2}$	24.50
S97M	$2.33 \times 10^6$	$2.90 \times 10^{-2}$	12.40
S97Q	$7.30 \times 10^5$	$5.98 \times 10^{-3}$	8.19
S97N	$8.95 \times 10^5$	$4.11 \times 10^{-3}$	4.59
S97H	$8.39 \times 10^5$	$2.48 \times 10^{-3}$	3.00
S97T	$4.63 \times 10^5$	$1.16 \times 10^{-3}$	2.51
S97V	$3.04 \times 10^6$	$3.58 \times 10^{-3}$	1.18
S97L	$7.50 \times 10^5$	$6.90 \times 10^{-4}$	0.92
S97I	$2.10 \times 10^6$	$1.80 \times 10^{-3}$	0.87
S97K	$1.45 \times 10^6$	$5.78 \times 10^{-3}$	0.40

TABLE VIII

5

BIAcore Analysis of "AT" Abs  
Substituted at N98 of Heavy Chain CDR3

	$K_a$ ( $M^{-1}s^{-1}$ )	$K_d$ ( $s^{-1}$ )	$K_D$ (nM)
N98K			No detectable binding
N98F	$3.70 \times 10^3$	$9.90 \times 10^{-2}$	26600
N98Y	$2.72 \times 10^5$	$1.12 \times 10^{-2}$	41.30
N98M	$2.96 \times 10^5$	$8.14 \times 10^{-2}$	27.50
N98T	$2.46 \times 10^5$	$6.62 \times 10^{-2}$	26.90
N98G	$2.30 \times 10^5$	$5.59 \times 10^{-3}$	24.30
N98I	$5.68 \times 10^5$	$4.36 \times 10^{-3}$	7.60
N98W	$2.62 \times 10^5$	$7.68 \times 10^{-3}$	2.93
N98E	$2.72 \times 10^5$	$3.59 \times 10^{-3}$	1.32
N98P	$1.51 \times 10^5$	$1.71 \times 10^{-3}$	1.13
N98H	$2.20 \times 10^5$	$2.34 \times 10^{-3}$	1.06
N98L	$1.48 \times 10^5$	$1.22 \times 10^{-3}$	0.82
N98Q	$1.86 \times 10^5$	$1.51 \times 10^{-3}$	0.81
N98S	$2.67 \times 10^5$	$1.54 \times 10^{-3}$	0.58
N98D	$6.85 \times 10^5$	$3.12 \times 10^{-3}$	0.46
N98V	$4.56 \times 10^5$	$1.97 \times 10^{-4}$	0.43
N98R	$3.59 \times 10^5$	$1.08 \times 10^{-3}$	0.30

10

While the present invention has been described in terms of preferred embodiments, it was understood that variations and modifications will occur to those skilled in the art. Therefore, it was intended that the appended claims cover all such

15

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equivalent variations which would come within the scope of the invention as claimed.

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## WHAT IS CLAIMED IS:

1. An antibody or antigen binding domain,  
or fragment, variant or derivative thereof, which binds  
5 to an osteoprotegerin binding protein and is an  
antagonist antibody.
2. The antibody of Claim 1 wherein the  
osteoprotegerin binding protein is mammalian  
10 osteoprotegerin binding protein.
3. The antibody of Claim 2 wherein the  
osteoprotegerin binding protein is human  
osteoprotegerin binding protein or an immunogenic  
15 fragment thereof.
4. The antibody of Claim 3 wherein the  
immunogenic fragment comprises at least part of the  
extracellular domain of a human osteoprotegerin binding  
20 protein.
5. The antibody of Claim 1 which inhibits  
the binding of osteoprotegerin binding protein to  
osteoclast differentiation and activation receptor.  
25
6. The antibody of Claim 1 which inhibits  
the formation or activation of osteoclasts.
7. The antibody of Claim 1 which inhibits  
30 bone resorption.
8. The antibody of Claim 1 which is  
selected from the group consisting of Fv, scFv, Fab,  
Fab' and F(ab')<sub>2</sub>.

35

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9. The antibody of Claim 1 which is a human antibody.

10. An antibody or antigen binding domain  
5 which comprises:

(a) a Fab heavy chain amino acid sequence as shown in Figure 9 (SEQ ID NO: 51) or Figure 10 (SEQ ID NO: 53);

(b) a heavy chain amino acid sequence  
10 comprising conservative amino acid substitutions of the sequence in (a);

(c) a heavy chain amino acid sequence which is at least about 80% identical to the sequence in (a);  
or

15 (d) a fragment or derivative of (a), (b) or (c);

wherein the antibody or antigen binding domain binds selectively to OPGbp.

20 11. The antibody of Claim 10 further comprising a kappa or lambda light chain.

12. The antibody of Claim 10 further comprising an human Fc region.

25 13. An antibody or antigen binding domain which recognizes an epitope on human OPGbp recognized by an antibody or antigen binding domain comprising the Fab heavy chain amino acid sequence as shown in Figure  
30 9 (SEQ ID NO: 51) or Figure 10 (SEQ ID NO: 53) and Fab light amino acid sequence as shown in Figure 5 (SEQ ID NO: 43) or Figure 6 (SEQ ID NO: 45)

14. An antibody or antigen binding domain  
35 comprising a variable light ( $V_L$ ) chain and a variable heavy ( $V_H$ ) chain:

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wherein each  $V_1$  chain comprises CDR amino acid sequences designated  $CDR1(V_1)$ ,  $CDR2(V_1)$  and  $CDR3(V_1)$  separated by framework amino acid sequences,  $CDR1(V_1)$  being selected from the group consisting of:

- 5        RASQISIRYLN    (SEQ ID NO: 01);  
          RASQSVGSYLA    (SEQ ID NO: 02);  
          RASQSVSSSSLA (SEQ ID NO: 03); and  
          SQDALPKQY        (SEQ ID NO: 04);

$CDR2(V_1)$  being selected from the group consisting of:

- 10       GASSLQS    (SEQ ID NO: 05);  
          DATNRAT    (SEQ ID NO: 06);  
          GASSRAT    (SEQ ID NO: 07); and  
          EDSERPS    (SEQ ID NO: 08);

and  $CDR3(V_1)$  being selected from the group consisting of:

- 15       QHTRA            (SEQ ID NO: 09);  
          QHRRT            (SEQ ID NO: 10);  
          QQYGA            (SEQ ID NO: 11); and  
          QSTDSSGTYVV    (SEQ ID NO: 12);

20       wherein  $CDR1(V_1)$ ,  $CDR2(V_1)$  and  $CDR3(V_1)$  are selected independently of each other; and

wherein each  $V_h$  chain comprises CDR amino acid sequences designated  $CDR1(V_h)$ ,  $CDR2(V_h)$  and  $CDR3(V_h)$  separated by framework amino acid sequences,  $CDR1(V_h)$  being selected from the group consisting of:

- 25       NYAIH    (SEQ ID NO: 13);  
          NYPMH    (SEQ ID NO: 14); and  
          DYAMH    (SEQ ID NO: 15)

$CDR2(V_h)$  being selected from the group consisting of:

- 30       WINAGNGNTKFSQKFQG (SEQ ID NO: 16);  
          VISYDGNNKYYADSVKG (SEQ ID NO: 17); and  
          GISWNSGRIGYADSVKG (SEQ ID NO: 18)

$CDR3(V_h)$  being selected from the group consisting of:

- 35       DSSNMVRGIIIAYYFDY (SEQ ID NO: 19);  
          GGGGFDY                (SEQ ID NO: 20); and  
          GGSTSARYSSGWYY        (SEQ ID NO: 21)



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wherein CDR1(V<sub>h</sub>), CDR2(V<sub>h</sub>) and CDR3(V<sub>h</sub>) are selected independently of each other.

15 15. The antibody of Claim 14 comprising a variable light (V<sub>l</sub>) chain and a variable heavy (V<sub>h</sub>) chain wherein:

the V<sub>l</sub> chain comprises CDR1 having the sequence RASQSI~~S~~RYLN (SEQ ID NO: 01), CDR2 having the sequence GASSLQS (SEQ ID NO: 05), and CDR3 having the sequence QHTRA (SEQ ID NO: 09); and

the V<sub>h</sub> chain comprises CDR1 having the sequence NYAIH (SEQ ID NO: 13), CDR2 having the sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3 having the sequence DSSNMVRGIIIIAYYFDY (SEQ ID NO: 19);

15 wherein CDR1, CDR2 and CDR3 on each V<sub>l</sub> and V<sub>h</sub> chain are separated by framework amino acid sequences.

20 16. The antibody of Claim 14 or 15 further comprising a human Fc region.

17. An antibody comprising a variable light (V<sub>l</sub>) chain and a variable heavy (V<sub>h</sub>) chain wherein:

the V<sub>l</sub> chain comprises a rearranged or somatic variant of the germline sequence of Figure 19 (SEQ ID NO: 66); and

25 the V<sub>h</sub> chain comprises a rearranged or somatic variant of the germline sequence of Figure 16 (SEQ ID NO: 59); and the antibody binds selectively to an osteoprotegerin binding protein.

30

18. An antibody comprising a variable light (V<sub>l</sub>) chain and a variable heavy (V<sub>h</sub>) chain wherein:

the V<sub>l</sub> chain comprises a rearranged or somatic variant of the germline sequence of Figure 20 (SEQ ID NO: 68); and

35

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the  $V_h$  chain comprises a rearranged or somatic variant of the germline sequence of Figure 16 (SEQ ID NO: 59); and the antibody binds selectively to an osteoprotegerin binding protein.

5

19. An antibody comprising a variable light ( $V_L$ ) chain and a variable heavy ( $V_h$ ) chain wherein:

the  $V_L$  chain comprises a rearranged or somatic variant of the germline sequence of Figure 21 (SEQ ID NO: 70); and

the  $V_h$  chain comprises a rearranged or somatic variant of the germline sequence of Figure 17 (SEQ ID NO: 62); and the antibody binds selectively to an osteoprotegerin binding protein.

15

20. An antibody comprising a variable light ( $V_L$ ) chain and a variable heavy ( $V_h$ ) chain wherein:

the  $V_L$  chain comprises a rearranged or somatic variant of the germline sequence of Figure 22 (SEQ ID NO: 72); and

the  $V_h$  chain comprises a rearranged or somatic variant of the germline sequence of Figure 18 (SEQ ID NO: 64); and the antibody binds selectively to an osteoprotegerin binding protein.

25

21. The antibody of Claim 1 which is a monoclonal antibody, a humanized antibody, a bispecific antibody, a single chain antibody, or a heteroantibody.

30

22. An isolated nucleic acid molecule encoding the antibody of any of Claims 1, 10, 13, 14, 15, 17, 18, 19, 20 or 21.

23. An expression vector comprising the nucleic acid molecule of Claim 22.

35

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24. A host cell comprising the expression vector of Claim 23.

25. The host cell of Claim 24 which is a CHO cell.

26. A method of producing an antibody comprising culturing the host cell of Claim 25 under conditions which allow expression of the nucleic acid molecule.

27. The antibody of Claims 1, 10, 13, 14, 15, 17, 18, 19, 20 or 21 wherein the IgG isotype is selected from IgG, IgM, IgA, IgE and IgD.

28. The antibody of Claim 27 wherein the isotype is IgG<sub>1</sub>, IgG<sub>2</sub>, IgG, or IgG<sub>4</sub>.

29. A composition comprising the antibody or antigen binding domain, or fragment, variant or derivative thereof, of any of Claims 1, 10, 13, 14, 15, 17, 18, 19, 20 or 21 and a pharmaceutically acceptable carrier.

30. A method of inhibiting osteoclast formation or activation comprising administering to a mammal an effective amount of the composition of Claim 29.

31. A method of inhibiting bone resorption comprising administering to a mammal an effective amount of the composition of Claim 29.

32. A method of preventing or treating loss of bone mass comprising administering to a mammal a

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therapeutically or prophylactically effective amount of the composition of Claim 29.

33. The method of Claim 32 wherein loss of  
5 bone mass results from osteoporosis, metastasis of cancer to bone; rheumatoid arthritis, hypercalcemia of malignancy, and steroid-induced osteoporosis.

34. The method of any of Claims 30, 31, 32  
10 or 33 further comprising administering a composition comprising at least one additional bone anti-resorptive agent.

35. The method of Claim 34 wherein the anti-  
15 resorptive agent is estrogen, a bisphosphonate, or a selective estrogen receptor modulator.

36. The method of any of Claims 30, 31, 32  
or 33 further comprising administering a composition  
20 comprising an anabolic agent for bone.

37. The method of Claim 36 wherein the  
anabolic agent is parathyroid hormone or a complex of  
insulin-like growth factor and insulin-like growth  
25 factor binding protein.

38. The method of any of Claims 30, 31, 32  
or 33 further comprising administering an interleukin-1  
inhibitor or a tumor necrosis factor-alpha inhibitor.  
30

39. A method of preventing or treating tumor  
cell growth in bone comprising administering to a  
mammal an effective amount of the composition of Claim  
29.

35

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40. An antibody or antigen binding domain which recognizes a DE epitope on human osteoprotegerin binding protein (OPGbp).

5 41. An antibody or antigen binding domain which recognizes a DE epitope on human OPGbp.

42. The antibody or antigen binding domain of Claim 40 or 41 wherein the DE epitope comprises the  
10 sequence DLATE.

43. An antibody or antigen binding domain which binds to murine OPGbp comprising the amino acid substitutions S229D, V230L, P231A, and D233E, but does  
15 not bind to murine OPGbp lacking said substitutions.

44. The antibody or antigen binding domain of Claims 40, 41, 42 or 43 which inhibits the formation of activation of osteoclasts.  
20

45. The antibody or antigen binding domain of Claims 40, 41, 42 or 43 which inhibits bone resorption.

25 46. An antibody comprising a variable light (Vl) and a variable heavy (Vh) chain, wherein the Vl chain comprises CDR1 having the sequence RASQSISRVLN (SEQ ID NO: 01), CDR2 having the sequence GASSLQS (SEQ ID NO: 05), and CDR3 having the sequence QHTRA (SEQ ID NO: 09), wherein CDR1, CDR2 and CDR3 on each Vl chain  
30 are separated by framework amino acid sequences,

and wherein the antibody binds selectively to an osteoprotegerin binding protein.

35 47. An antibody comprising a variable light (Vl) chain and a variable heavy (Vh) chain wherein the

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V<sub>h</sub> chain comprises CDR1 having the sequence NYAIH (SEQ ID NO: 13), CDR2 having the sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3 having the sequence DSSNMVRGIIIAYYFDY (SEQ ID NO: 19), wherein CDR1, CDR2 and CDR3 on each V<sub>h</sub> chain are separated by framework amino acid sequences,  
 and wherein the antibody binds selectively to an osteoprotegerin binding protein.

48. An antibody comprising a variable light (V<sub>l</sub>) chain and a variable heavy (V<sub>h</sub>) chain wherein:  
 the V<sub>l</sub> chain comprises CDR1 having the sequence RASQISRYLN (SEQ ID NO: 01), CDR2 having the sequence GASSLQS (SEQ ID NO: 05), and CDR3 having the sequence QHTRA (SEQ ID NO: 09); and  
 the V<sub>h</sub> chain comprises CDR1 having the sequence NYAIH (SEQ ID NO: 13), CDR2 having the sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3 having the sequence selected from the group consisting of:

XSSNMVRGIIIAYYFDY (SEQ ID NO: 80);  
 DXSNMVRGIIIAYYFDY (SEQ ID NO: 81);  
 DSXNMVRGIIIAYYFDY (SEQ ID NO: 82);  
 DSSXMVRGIIIAYYFDY (SEQ ID NO: 83);  
 DSSNXVRGIIIAYYFDY (SEQ ID NO: 84);  
 DSSNMXRGIIIAYYFDY (SEQ ID NO: 85);  
 DSSNMVXGIIIAYYFDY (SEQ ID NO: 86);  
 DSSNMVRXIIIAYYFDY (SEQ ID NO: 87);  
 DSSNMVRGXIIAYYFDY (SEQ ID NO: 88);  
 DSSNMVRGIXIAYYFDY (SEQ ID NO: 89);  
 DSSNMVRGIIIXAYYFDY (SEQ ID NO: 90);  
 DSSNMVRGIIIXYYFDY (SEQ ID NO: 91);  
 DSSNMVRGIIIAXYFDY (SEQ ID NO: 92);  
 DSSNMVRGIIIAYXFDY (SEQ ID NO: 93);  
 DSSNMVRGIIIAYYXDY (SEQ ID NO: 94);  
 DSSNMVRGIIIAYYFXY (SEQ ID NO: 95); and



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DSSNMVRGIIIAYYFDX (SEQ ID NO: 96);  
 wherein CDR1, CDR2 and CDR3 on each V<sub>L</sub> and V<sub>H</sub> chain are  
 separated by framework amino acid sequences and X is  
 any amino acid different from the amino acid normally  
 5 resident at that position;

and wherein the antibody binds selectively to  
 an osteoprotegerin binding protein.

49. An antibody comprising a variable light  
 10 (V<sub>L</sub>) chain and a variable heavy (V<sub>H</sub>) chain wherein:  
 the V<sub>H</sub> chain comprises CDR1 having the  
 sequence NYAIH (SEQ ID NO: 13), CDR2 having the  
 sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3  
 having the sequence selected from the group consisting  
 15 of:

XSSNMVRGIIIAYYFDY (SEQ ID NO: 80);  
 DXSNMVRGIIIAYYFDY (SEQ ID NO: 81);  
 DSXNMVRGIIIAYYFDY (SEQ ID NO: 82);  
 DSSXMVRGIIIAYYFDY (SEQ ID NO: 83);  
 20 DSSNXVRGIIIAYYFDY (SEQ ID NO: 84);  
 DSSNMXRGIIIAYYFDY (SEQ ID NO: 85);  
 DSSNMVXGIIIAYYFDY (SEQ ID NO: 86);  
 DSSNMVRXIIIAYYFDY (SEQ ID NO: 87);  
 DSSNMVRGXIIIAYYFDY (SEQ ID NO: 88);  
 25 DSSNMVRGIXIAYYFDY (SEQ ID NO: 89);  
 DSSNMVRGIIXAYYFDY (SEQ ID NO: 90);  
 DSSNMVRGIIIXYYFDY (SEQ ID NO: 91);  
 DSSNMVRGIIIAXYFDY (SEQ ID NO: 92);  
 DSSNMVRGIIIAYXFDY (SEQ ID NO: 93);  
 30 DSSNMVRGIIIAYYXDY (SEQ ID NO: 94);  
 DSSNMVRGIIIAYYFXY (SEQ ID NO: 95); and  
 DSSNMVRGIIIAYYFDX (SEQ ID NO: 96);

wherein CDR1, CDR2 and CDR3 on each V<sub>L</sub> and V<sub>H</sub> chain are  
 separated by framework amino acid sequences and X is  
 35 any amino acid different from the amino acid normally  
 resident at that position;

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and wherein the antibody binds selectively to an osteoprotegerin binding protein.

50. An antibody comprising a variable light (V<sub>L</sub>) chain and a variable heavy (V<sub>H</sub>) chain wherein:  
the V<sub>L</sub> chain comprises CDR1 having the sequence RASQISRYLN (SEQ ID NO: 01), CDR2 having the sequence GASSLQS (SEQ ID NO: 05), and CDR3 having the sequence QHTRA (SEQ ID NO: 09); and  
the V<sub>H</sub> chain comprises CDR1 having the sequence NYAIH (SEQ ID NO: 13), CDR2 having the sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3 having one or more amino acid substitutions in the sequence DSSNMVRGIIIAYYFDY (SEQ ID NO: 19);  
wherein CDR1, CDR2 and CDR3 on each V<sub>L</sub> and V<sub>H</sub> chain are separated by framework amino acid sequences, and wherein the antibody binds selectively to an osteoprotegerin binding protein.

51. An antibody comprising a variable light (V<sub>L</sub>) chain and a variable heavy (V<sub>H</sub>) chain wherein:  
the V<sub>H</sub> chain comprises CDR1 having the sequence NYAIH (SEQ ID NO: 13), CDR2 having the sequence WINAGNGNTKFSQKFQG (SEQ ID NO: 16), and CDR3 having one or more amino acid substitutions in the sequence DSSNMVRGIIIAYYFDY (SEQ ID NO: 19);  
wherein CDR1, CDR2 and CDR3 on each V<sub>L</sub> and V<sub>H</sub> chain are separated by framework amino acid sequences, and wherein the antibody binds selectively to an osteoprotegerin binding protein.

52. An antibody or antigen binding domain which binds selectively to human OPGbp with a dissociation constant (K<sub>D</sub>) of about 1nM or less.

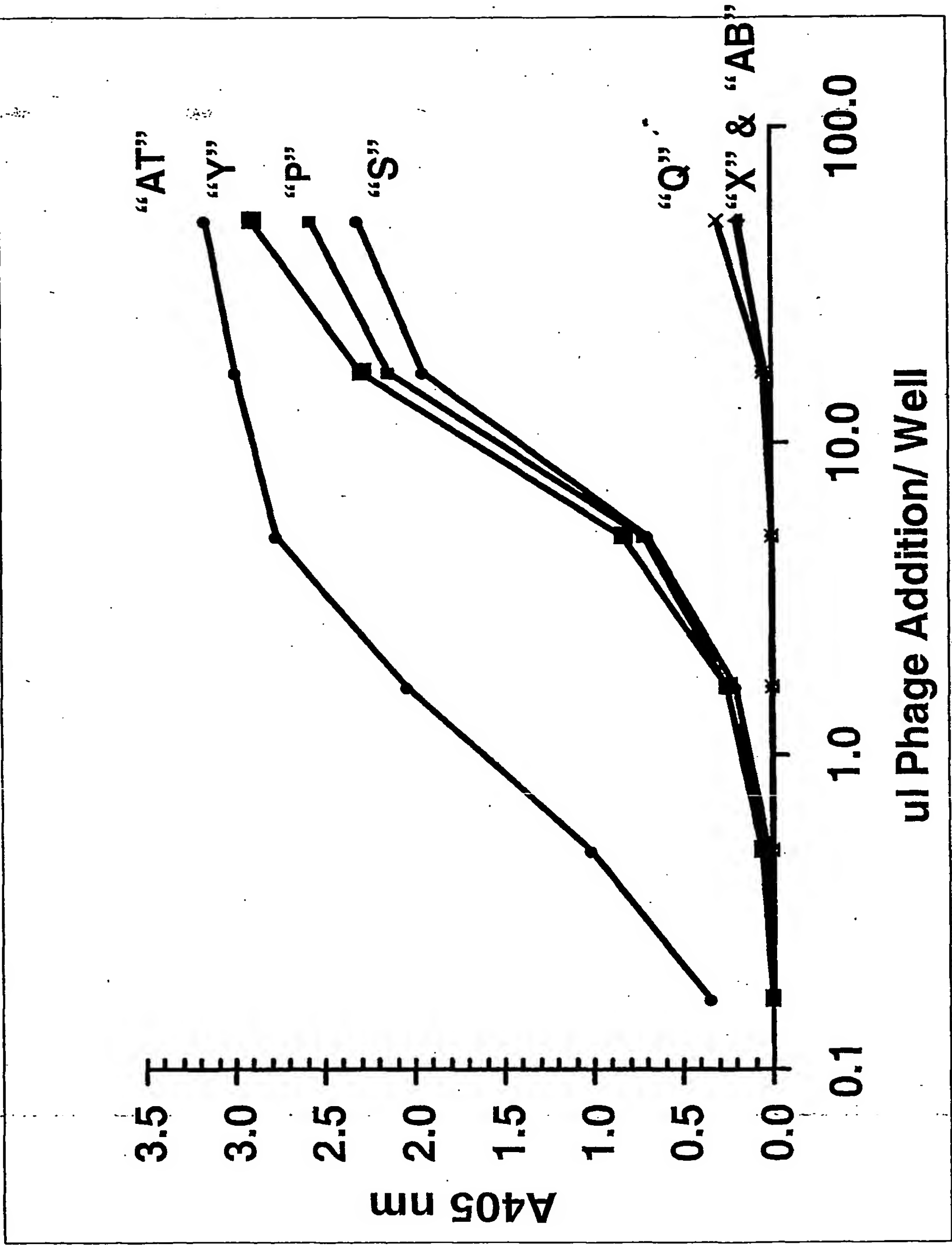
35

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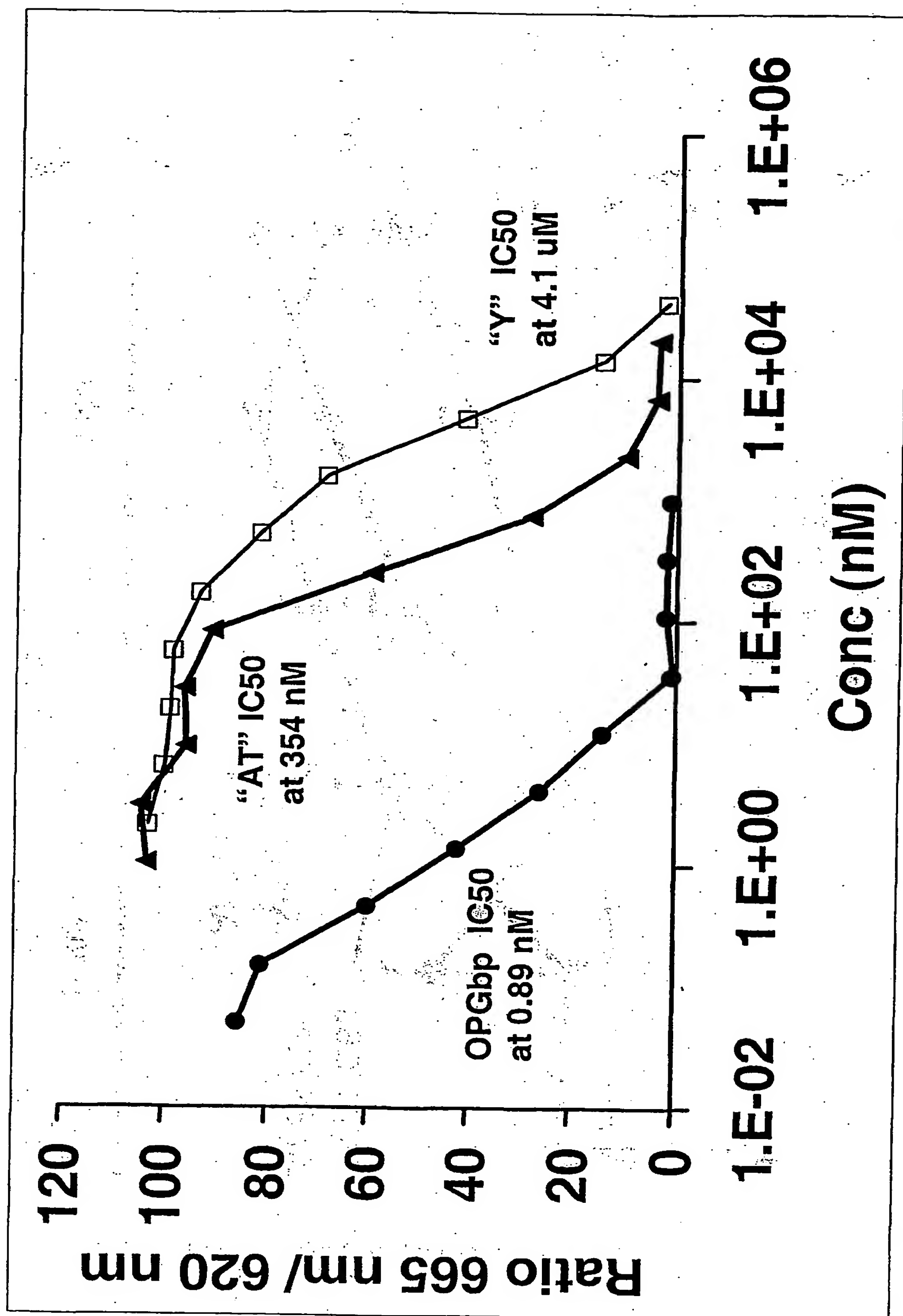
53.. An antibody or antigen binding domain which binds selectively to human OPGbp with a dissociation rate constant (kd) of about  $3 \times 10^{-3}$  or less.

5

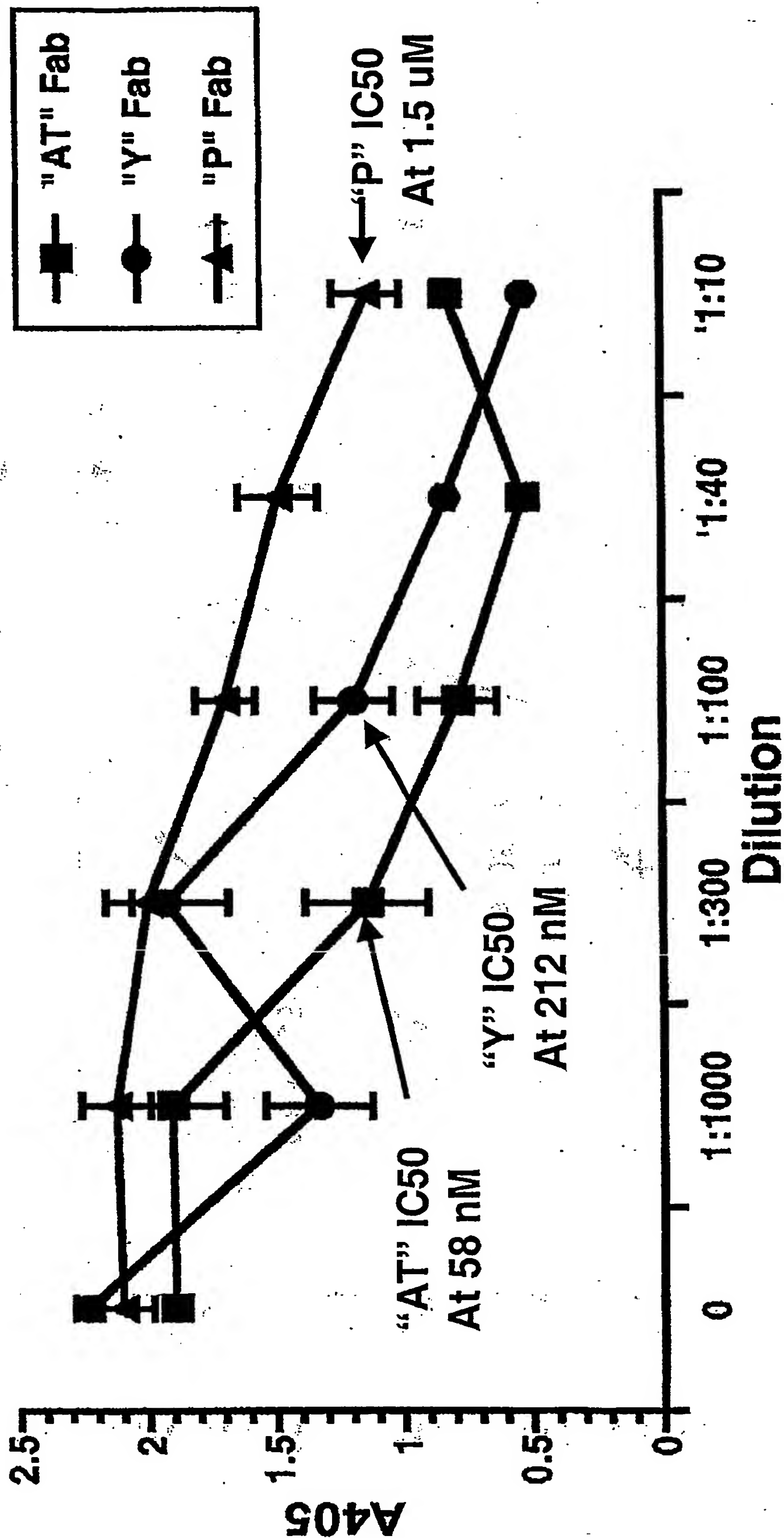
Figure 1  
Phage ELISA



**Figure 2**  
**Inhibition of OPGbp/ODAR Interaction by Fabs "AT" and "Y"**

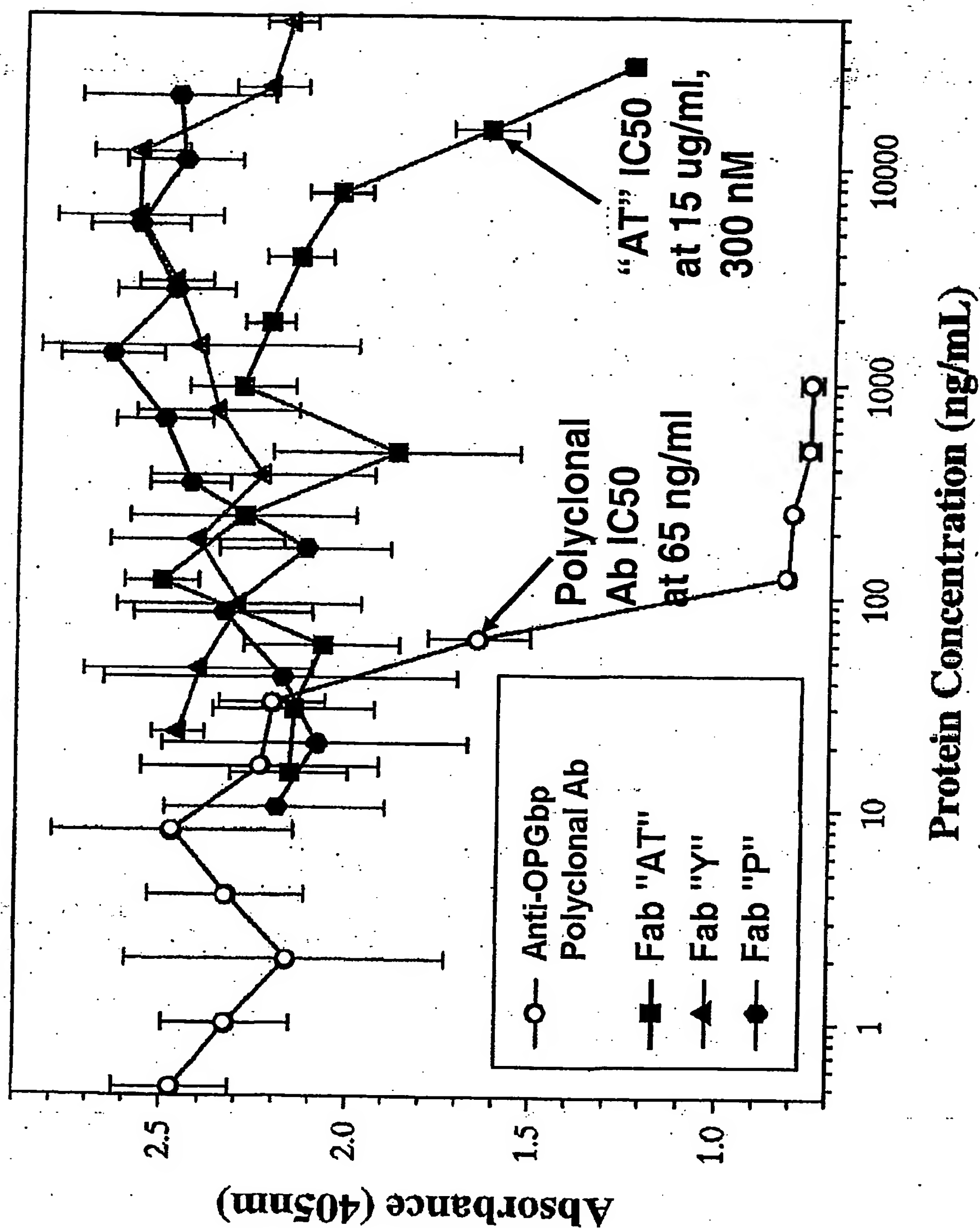


**Figure 3**  
**Bone Marrow Assay**  
**Fabs "AT" "Y" "P"**





**Figure 4**  
**Raw Cell Bioassay**  
**Fabs "AT" "Y" and "P"**



## Figure 5

AT LIGHT

TCT	CAC	AGT	GCA	CTT	GAA	ATT	GTG	ATG	ACG	CAG	TCT	CCA	TCC	TCC	CTG	48
Ser	His	Ser	Ala	Leu	Glu	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	
1				5				10						15		
TCT	GCG	TCT	GTT	GGA	GAC	AGA	GTG	ACC	ATC	ACT	TGC	CGG	GCA	AGT	CAG	96
Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	
			20					25					30			
AGC	ATT	AGC	AGA	TAT	TTA	AAT	TGG	TAT	CAG	CTT	AAA	CCA	GGG	AAA	GCC	144
Ser	Ile	Ser	Arg	Tyr	Leu	Asn	Trp	Tyr	Gln	Leu	Lys	Pro	Gly	Lys	Ala	
		35					40					45				
CCT	AGG	CTC	CTG	ATC	TAT	GGT	GCA	TCC	AGT	TTG	CAA	AGT	GGA	GTC	CCA	192
Pro	Arg	Leu	Leu	Ile	Tyr	Gly	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	
	50					55					60					
TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT	GGG	GCA	GAG	TTC	ACT	CTC	ACC	ATC	240
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Ala	Glu	Phe	Thr	Leu	Thr	Ile	
65				70				75							80	
AGC	AGT	CTA	CAA	CCT	GAA	GAC	ATT	GCC	ACT	TAC	TAC	TGT	CAA	CAC	ACT	288
Ser	Ser	Leu	Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Thr	
			85					90						95		
CGG	GCG	TTC	GGC	CAA	GGG	ACC	AAG	GTT	GAA	ATC	AAG	CGA	ACT	GTG	GCT	336
Arg	Ala	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	
			100					105					110			
GCA	CCA	TCT	GTC	TTC	ATC	TTC	CCG	CCA	TCT	GAT	GAG	CAG	TTG	AAA	TCT	384
Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
		115					120					125				
GGA	ACT	GCC	TCT	GTT	GTG	TGC	CTG	CTG	AAT	AAC	TTC	TAT	CCC	AGA	GAG	432
Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	
	130					135					140					
GCC	AAA	GTA	CAG	TGG	AAG	GTG	GAT	AAC	GCC	CTC	CAA	TCG	GGT	AAC	TCC	480
Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	
145				150				155							160	
CAG	GAG	AGT	GCC	ACA	GAG	CAG	GAC	AGC	AAG	GAC	AGC	ACC	TAC	AGC	CTC	528
Gln	Glu	Ser	Ala	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	
			165					170						175		
AGC	AGC	ACC	CTG	ACG	CTG	AGC	AAA	GCA	GAC	TAC	GAG	AAA	CAC	AAA	GTC	576
Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
			180					185					190			
TAC	GCC	TGC	GAA	GTC	ACC	CAT	CAG	GGC	CTG	AGC	TCG	CCC	GTC	ACA	AAG	624
Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	
		195					200					205				
AGC	TTC	AAC	AGG	GGA	GAG	TGT										645
Ser	Phe	Asn	Arg	Gly	Glu	Cys										
	210					215										

## Figure 6

Y LIGHT

TCT	CAC	AGT	GCA	CTT	GAA	ATT	GTG	CTG	ACT	CAG	TCT	CCA	GCC	ACC	CTG	48
Ser	His	Ser	Ala	Leu	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	
1				5				10						15		
TCT	TTT	TCT	CCG	GGT	GAA	AGA	GCC	ACC	CTC	TCC	TGC	AGG	GCC	AGT	CAG	96
Ser	Phe	Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	
			20					25					30			
AGT	GTT	GGC	AGC	TAC	TTA	GCC	TGG	TAC	CAG	CAG	AGA	CCT	GGC	CAG	GCT	144
Ser	Val	Gly	Ser	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Arg	Pro	Gly	Gln	Ala	
		35					40					45				
CCC	AGG	CCC	CTC	ATC	TAT	GAT	GCA	ACC	AAC	AGG	GCC	ACT	GGC	ATC	CCA	192
Pro	Arg	Pro	Leu	Ile	Tyr	Asp	Ala	Thr	Asn	Arg	Ala	Thr	Gly	Ile	Pro	
	50					55					60					
ACC	AGG	TTC	AGT	GGC	AGT	GGG	TCT	GGG	ACA	GAC	TTC	ACT	CTC	ACC	ATC	240
Thr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	
65				70						75					80	
AGC	AGC	CTA	GAG	CCT	GAA	GAT	TTT	GCA	ACT	TAT	TAC	TGT	CAA	CAC	CGA	288
Ser	Ser	Leu	Glu	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Arg	
				85					90					95		
AGG	ACT	TTT	GGC	CGG	GGG	ACC	AAG	TTG	GAG	ATC	AAA	CGA	ACT	GTG	GCT	336
Arg	Thr	Phe	Gly	Arg	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	
			100					105					110			
GCA	CCA	TCT	GTC	TTC	ATC	TTC	CCG	CCA	TCT	GAT	GAG	CAG	TTG	AAA	TCT	384
Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
		115					120					125				
GGA	ACT	GCC	TCT	GTT	GTG	TGC	CTG	CTG	AAT	AAC	TTC	TAT	CCC	AGA	GAG	432
Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	
	130					135					140					
GCC	AAA	GTA	CAG	TGG	AAG	GTG	GAT	AAC	GCC	CTC	CAA	TCG	GGT	AAC	TCC	480
Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	
145					150				155						160	
CAG	GAG	AGT	GTC	ACA	GAG	CAG	GAC	AGC	AAG	GAC	AGC	ACC	TAC	AGC	CTC	528
Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	
				165					170					175		
AGC	AGC	ACC	CTG	ACG	CTG	AGC	AAA	GCA	GAC	TAC	GAG	AAA	CAC	AAA	GTC	576
Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
			180					185					190			
TAC	GCC	TGC	GAA	GTC	ACT	CAT	CAG	GGC	CTG	AGC	TCG	CCC	GTC	ACA	AAG	624
Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	
		195					200					205				
AGC	TTC	AAC	AGG	GGA	GAG	TGT										645
Ser	Phe	Asn	Arg	Gly	Glu	Cys										
	210					215										

## Figure 7

P LIGHT

CAC	AGT	GCA	CTT	GAA	ATT	GTG	ATG	ACA	CAG	TCT	CCA	GGC	ACC	CTG	TCT	48
His	Ser	Ala	Leu	Glu	Ile	Val	Met	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	
1				5					10					15		
TTG	TCT	CCA	GGG	GAA	AGA	GCC	ACC	CTC	TCC	TGC	AGG	GCC	AGT	CAG	AGT	96
Leu	Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	
			20					25					30			
GTT	AGC	AGC	AGC	TCC	TTA	GCC	TGG	TAC	CAG	CAG	AAA	CCT	GGC	CAG	GCT	144
Val	Ser	Ser	Ser	Ser	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	
			35				40					45				
CCC	AGG	CTC	CTC	ATC	TAT	GGT	GCA	TCC	AGC	AGG	GCC	ACT	GGC	ATC	CCA	192
Pro	Arg	Leu	Leu	Ile	Tyr	Gly	Ala	Ser	Ser	Arg	Ala	Thr	Gly	Ile	Pro	
	50					55					60					
GAC	AGG	TTC	AGT	GGC	AGT	GGG	TCT	GGG	ACA	GAC	TTC	ACT	CTC	ACC	ATC	240
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	
65				70					75						80	
AGC	AGA	CTG	GAG	CCT	GAA	GAT	TTT	GCA	GTG	TAT	TAC	TGT	CAG	CAG	TAT	288
Ser	Arg	Leu	Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	
				85					90					95		
GGT	GCT	TTC	GGC	GGA	GGG	ACC	AAG	GTG	GAG	ATC	AAA	CGA	ACT	GTG	GCT	336
Gly	Ala	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	
			100					105					110			
GCA	CCA	TCT	GTC	TTC	ATC	TTC	CCG	CCA	TCT	GAT	GAG	CAG	TTG	AAA	TCT	384
Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
			115				120					125				
GGA	ACT	GCC	TCT	GTT	GTG	TGC	CTG	CTG	AAT	AAC	TTC	TAT	CCC	AGA	GAG	432
Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	
	130					135					140					
GCC	AAA	GTA	CAG	TGG	AAG	GTG	GAT	AAC	GCC	CTC	CAA	TCG	GGT	AAC	TCC	480
Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	
145					150				155						160	
CAG	GAG	AGT	GTC	ACA	GAG	CAG	GAC	AGC	AAG	GAC	AGC	ACC	TAC	AGC	CTC	528
Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	
				165					170					175		
AGC	AGC	ACC	CTG	ACG	CTG	AGC	AAA	GCA	GAC	TAC	GAG	AAA	CAC	AAA	GTC	576
Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
			180					185					190			
TAC	GCC	TGC	GAA	GTC	ACC	CAT	CAG	GGC	CTG	AAC	TCG	CCC	GTC	ACA	AAG	624
Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Asn	Ser	Pro	Val	Thr	Lys	
		195					200					205				
AGC	TTC	AAC	AGG	GGA	GAG	TGT										645
Ser	Phe	Asn	Arg	Gly	Glu	Cys										
	210					215										

## Figure 8

S LIGHT

TCT CAC AGT GCA CAG TCT GTG CTG ACT CAG CCA CCC TCG GTG TCA GTG Ser His Ser Ala Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val 1 5 10 15	48
TCC CCA GGA CAG ACG GCC ACG ATC ACC TGC TCT GGA GAT GCA TTG CCA Ser Pro Gly Gln Thr Ala Thr Ile Thr Cys Ser Gly Asp Ala Leu Pro 20 25 30	96
AAG CAA TAT GTT TAT TGG TAC CGG CAG AAG CCA GGC CAG GCC CCT CTA Lys Gln Tyr Val Tyr Trp Tyr Arg Gln Lys Pro Gly Gln Ala Pro Leu 35 40 45	144
TTG GTG ATA TAT GAA GAC AGT GAG AGG CCC TCA GGG ATC CCT GAA CGA Leu Val Ile Tyr Glu Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg 50 55 60	192
TTC TCT GGC TCC AGT TCA GGG ACT GAA GTC ACG TTG AGT ATC AGT GGA Phe Ser Gly Ser Ser Ser Gly Thr Glu Val Thr Leu Ser Ile Ser Gly 65 70 75 80	240
GTC CAG GCA GAA GAC GAG GCT GAC TAT TAT TGT CAA TCA ACA GAC AGC Val Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Thr Asp Ser 85 90 95	288
AGT GGG ACT TAT GTC GTC TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA Ser Gly Thr Tyr Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu 100 105 110	336
AGT CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC TCT Ser Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser 115 120 125	384
GAG GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp 130 135 140	432
TTC TAC CCG GGA GCC GTG ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro 145 150 155 160	480
GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC TCC AAA CAA AGC AAC AAC Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn 165 170 175	528
AAG TAC GCG GCC AGC AGC TAT CTG AGC CTG ACG CCT GAG CAG TGG AAG Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys 180 185 190	576
TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG CAT GAA GGG AGC ACC GTG Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val 195 200 205	624
GAG AAG ACA GTG GCC CCT ACA GAA TGT TCA Glu Lys Thr Val Ala Pro Thr Glu Cys Ser 210 215	654

## Figure 9

AT HEAVY

GCC	CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGG	GCT	GAG	GTG	AGG	AAG	CCT	GGG	48
Ala	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Arg	Lys	Pro	Gly	
1				5				10						15		
GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAG	GCT	TCT	GGA	TAC	GAC	TTC	AGT	AAT	96
Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Asp	Phe	Ser	Asn	
			20					25					30			
TAT	GCT	ATA	CAT	TGG	GTG	CGC	CAG	GCC	CCC	GGA	CAA	AGG	CTT	GAG	TGG	144
Tyr	Ala	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu	Glu	Trp	
		35					40					45				
ATG	GGA	TGG	ATC	AAC	GCT	GGC	AAT	GGG	AAC	ACA	AAA	TTT	TCA	CAG	AAG	192
Met	Gly	Trp	Ile	Asn	Ala	Gly	Asn	Gly	Asn	Thr	Lys	Phe	Ser	Gln	Lys	
	50					55					60					
TTC	CAG	GGC	AGA	ATC	ACC	GTT	ACC	AGG	GAC	ACA	GCC	GCG	AGC	ACA	GCC	240
Phe	Gln	Gly	Arg	Ile	Thr	Val	Thr	Arg	Asp	Thr	Ala	Ala	Ser	Thr	Ala	
65					70				75						80	
TAC	ATG	GAG	CTG	CGC	AGT	CTG	AGA	TCT	GAA	GAC	ACG	GCT	GTG	TAT	TAC	288
Tyr	Met	Glu	Leu	Arg	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	
				85					90					95		
TGT	GCG	AGA	GAT	TCC	TCA	AAT	ATG	GTT	CGG	GGA	ATT	ATT	ATA	GCG	TAC	336
Cys	Ala	Arg	Asp	Ser	Ser	Asn	Met	Val	Arg	Gly	Ile	Ile	Ile	Ala	Tyr	
			100					105					110			
TAT	TTT	GAC	TAC	TGG	GGC	CAG	GGC	ACC	CTG	GTC	ACC	GTC	TCA	AGC	GCC	384
Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	
		115					120					125				
TCC	ACC	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	432
Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	
	130					135					140					
ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	480
Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	
145					150				155						160	
CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC	CTG	ACC	AGC	GGC	528
Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	
				165					170					175		
GTC	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	CTC	TAC	TCC	CTC	576
Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	
			180					185					190			
AGC	AGC	GTA	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	ACC	TAC	624
Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	
		195					200					205				



## Figure 9 (con't)

ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA	672
Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	
210						215					220					
GTT	GAG	CCC	AAA	TCT	TGT											690
Val	Glu	Pro	Lys	Ser	Cys											
225					230											

Figure 10

Y HEAVY

GCC	GAG	GTC	CAG	CTG	GTG	CAG	TCT	GGG	GCT	GAG	GTG	AGG	AAG	CCT	GGG	48
Ala	Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Arg	Lys	Pro	Gly	
1				5				10						15		
GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAG	GCT	TCT	GGA	TAC	GAC	TTC	AGT	AAT	96
Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Asp	Phe	Ser	Asn	
			20					25					30			
TAT	GCT	ATA	CAT	TGG	GTG	CGC	CAG	GCC	CCC	GGA	CAA	AGG	CTT	GAG	TGG	144
Tyr	Ala	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu	Glu	Trp	
		35					40					45				
ATG	GGA	TGG	ATC	AAC	GCT	GGC	AAT	GGG	AAC	ACA	AAA	TTT	TCA	CAG	AAG	192
Met	Gly	Trp	Ile	Asn	Ala	Gly	Asn	Gly	Asn	Thr	Lys	Phe	Ser	Gln	Lys	
	50					55					60					
TTC	CAG	GGC	AGA	ATC	ACC	GTT	ACC	AGG	GAC	ACA	GCC	GCG	AGC	ACA	GCC	240
Phe	Gln	Gly	Arg	Ile	Thr	Val	Thr	Arg	Asp	Thr	Ala	Ala	Ser	Thr	Ala	
65					70					75					80	
TAC	ATG	GAG	CTG	CGC	AGT	CTG	AGA	TCT	GAA	GAC	ACG	GCT	GTG	TAT	TAC	288
Tyr	Met	Glu	Leu	Arg	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	
				85					90					95		
TGT	GCG	AGA	GAT	TCC	TCA	AAT	ATG	GTT	CGG	GGA	ATT	ATT	ATA	GCG	TAC	336
Cys	Ala	Arg	Asp	Ser	Ser	Asn	Met	Val	Arg	Gly	Ile	Ile	Ile	Ala	Tyr	
			100					105					110			
TAT	TTT	GAC	TAC	TGG	GGC	CAG	GGC	ACC	CTG	GTC	ACC	GTC	TCA	AGC	GCC	384
Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	
		115					120					125				
TCC	ACC	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	432
Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	
	130					135					140					
ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	480
Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	
145					150					155					160	
CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC	CTG	ACC	AGC	GGC	528
Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	
				165				170						175		
GTC	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	CTC	TAC	TCC	CTC	576
Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	
			180					185					190			
AGC	AGC	GTA	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	ACC	TAC	624
Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	
		195					200					205				

## Figure 10 (con't)

ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA	672
Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	
210						215					220					

GTT	GAG	CCC	AAA	TCT	TGT		690
Val	Glu	Pro	Lys	Ser	Cys		
225					230		

## Figure 11

P HEAVY

GCC	GAG	GTC	CAG	CTG	GTG	CAG	TCT	GGG	GGA	GGC	TTG	GTC	CAG	CCT	GGG	48
Ala	Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	
1				5				10						15		
GGG	TCC	CTG	AGA	CTC	TCC	TGT	TTA	GTG	TCT	GGA	TTC	ACC	TTC	AAT	AAC	96
Gly	Ser	Leu	Arg	Leu	Ser	Cys	Leu	Val	Ser	Gly	Phe	Thr	Phe	Asn	Asn	
			20					25					30			
TAT	CCT	ATG	CAC	TGG	GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGG	CTG	GAG	TGG	144
Tyr	Pro	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	
		35					40					45				
GTG	GCA	GTT	ATA	TCA	TAT	GAT	GGA	AAT	AAT	AAA	TAC	TAC	GCA	GAC	TCC	192
Val	Ala	Val	Ile	Ser	Tyr	Asp	Gly	Asn	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	
	50					55					60					
GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	AGA	GAC	AAT	TCC	AAG	AAC	ACG	CTG	240
Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	
65					70					75					80	
TAT	TTG	CAA	ATG	AAC	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCC	GTG	TAT	TAC	288
Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	
				85					90					95		
TGT	GCG	AGG	GGG	GGC	GGT	GGC	TTT	GAC	TAC	TGG	GGC	CAG	GGA	ACC	CTG	336
Cys	Ala	Arg	Gly	Gly	Gly	Gly	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	
			100					105					110			
GTC	ACC	GTC	TCA	AGC	GCC	TCC	ACC	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	384
Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	
		115					120					125				
GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	432
Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	
	130					135					140					
CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	480
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	
145					150					155					160	
GGC	GCC	CTG	ACC	AGC	GGC	GTC	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	528
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	
				165					170					175		
TCA	GGA	CTC	TAC	TCC	CTC	AGC	AGC	GTA	GTG	ACC	GTG	CCC	TCC	AGC	AGC	576
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	
			180					185					190			
TTG	GGC	ACC	CAG	ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	624
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	
		195					200					205				
ACC	AAG	GTG	GAC	AAG	AAA	GTT	GAG	CCC	AAA	TCT	TGT					660
Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys					
	210					215					220					

Figure 12

S HEAVY

GCC	GAG	GTG	CAG	CTG	CTG	GAG	TCT	GGG	GGA	GGC	TTG	GTA	CAA	CCT	GGC	48
Ala	Glu	Val	Gln	Leu	Leu	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	
1				5				10						15		
AGG	TCC	CTG	AGA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	TTC	ACC	TTT	GAT	GAT	96
Arg	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	Asp	
			20					25					30			
TAT	GCC	ATG	CAC	TGG	GTC	CGG	CAA	GCT	CCA	GGG	AAG	GGC	CTG	GAG	TGG	144
Tyr	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	
		35				40					45					
GTC	TCA	GGT	ATT	AGT	TGG	AAT	AGT	GGT	AGG	ATA	GGC	TAT	GCG	GAC	TCT	192
Val	Ser	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Arg	Ile	Gly	Tyr	Ala	Asp	Ser	
	50					55					60					
GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	AGA	GAC	AAC	GCC	AAG	AAC	TCC	CTG	240
Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	
65					70					75					80	
TAT	CTG	CAA	ATG	AAC	AGT	CTG	AGA	CCT	GAG	GAC	ACG	GCC	TTC	TAT	TAC	288
Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Pro	Glu	Asp	Thr	Ala	Phe	Tyr	Tyr	
				85				90						95		
TGT	GCA	AAA	GGG	GGT	TCT	ACA	AGC	GCG	AGG	TAT	AGC	AGT	GGC	TGG	TAC	336
Cys	Ala	Lys	Gly	Gly	Ser	Thr	Ser	Ala	Arg	Tyr	Ser	Ser	Gly	Trp	Tyr	
			100					105					110			
TAC	TGG	GGC	CAG	GGC	ACC	CTG	GTC	ACC	GTC	TCA	AGC	GCC	TCC	ACC	AAG	384
Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	
		115				120						125				
GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	432
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	
	130					135					140					
GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	480
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	
145					150					155					160	
GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC	CTG	ACC	AGC	GGC	GTC	CAC	ACC	528
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	
				165				170						175		
TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	CTC	TAC	TCC	CTC	AGC	AGC	GTA	576
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	
			180					185					190			
GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	ACC	TAC	ATC	TGC	AAC	624
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	
		195					200					205				
GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA	GTT	GAG	CCC	672
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	
	210					215					220					

Figure 12 (con't)

AAA TCT TGT  
Lys Ser Cys  
225

681



Figure 13  
Fab Amino Acid Sequence Comparison

		<u>Sequence Heavy Chain Matrix</u>			<u>Sequence Light Chain Matrix</u>				
SIMILARITY	IDENTITY	IDENTITY			IDENTITY				
		AT	Y	P	S	AT	Y	P	S
AT	---	99.6	76.9	72.3	AT	---	85.7	86.9	46.9
Y	100	---	78.6	72.8	Y	89.0	---	91.5	47.4
P	80.5	81.6	---	88.6	P	89.4	92.3	---	47.4
S	78.6	79.0	91.8	---	S	59.3	59.8	60.8	---

**Figure 14**  
**Fab CDRs Comparison**

Pattern		Heavy Chain		
	CDR1	CDR2	CDR3	
AT	NYA <sup>1</sup> I <sup>2</sup> H	WINAGNGNTKFSQKFQ <sup>3</sup> G	DSSNMVRGIIIA <sup>4</sup> YYFD <sup>5</sup> Y	
Y	NYA <sup>1</sup> I <sup>2</sup> H	WINAGNGNTKFSQKFQ <sup>3</sup> G	DSSNMVRGIIIA <sup>4</sup> YYFD <sup>5</sup> Y	
P	NYP <sup>1</sup> M <sup>2</sup> H	VISYDGN <sup>3</sup> NKYADSVK <sup>4</sup> G	GGGGFD <sup>5</sup> Y	
S	DYA <sup>1</sup> M <sup>2</sup> H	GISWNSGRIGYADSVK <sup>3</sup> G	GGTSARYSSGW <sup>4</sup> YY	
Pattern		Light Chain		
	CDR1	CDR2	CDR3	
AT	RASQSI <sup>1</sup> SR <sup>2</sup> YL <sup>3</sup> N	GASSLQ <sup>4</sup> S	QH <sup>5</sup> TR <sup>6</sup> A	
Y	RASQSV <sup>1</sup> GS <sup>2</sup> YL <sup>3</sup> A	DATN <sup>4</sup> RAT	QH <sup>5</sup> RR <sup>6</sup> T	
P	RASQSVSS <sup>1</sup> SS <sup>2</sup> SL <sup>3</sup> A	GASSR <sup>4</sup> AT	QQ <sup>5</sup> YGA <sup>6</sup>	
S	SGDALP <sup>1</sup> KQ <sup>2</sup> Y	EDSER <sup>4</sup> PS	QSTDSSGTY <sup>5</sup> VV	

**Figure 15**  
**Fab Classes**

Pattern	Heavy Chain		Light Chain	
	Family	V	Family	V
AT	VH1	1-03 3-10 JH4b	Vk1	012/02 JK1
Y	VH1	1-03 3-10 JH4b	Vk3	L6 JK2
P	VH3	3-30 * JH4b	Vk3	A27 JK4
S	VH3	3-09 6-19 JH4b	V <sub>L</sub> 3	** JL2/JL3

Figure 16

FABS "AT" AND "Y" HEAVY CHAIN SEQUENCE COMPARISON TO GERMLINE

H1												
FR1									CDR1		FR2	
-----									-----		-----	
1			2			3			4			
Locus	123456789012345678901234567890							1ab2345	67890123456789			
1-03	QVQLVQSGAEVKKPGASVKV	SCKASGYTFT						S--YAMH	WVRQAPGQRLEWMG			
"AT"	QVQLVQSGAEVRKPGASVKV	SCKASGYDFS						N YAIH	WVRQAPGQRLEWMG			
"Y"	EVQLVQSGAEVRKPGASVKV	SCKASGYDFS						N YAIH	WVRQAPGQRLEWMG			
H2												
-----									-----		-----	
CDR2						FR3						
-----						-----						
5		6				7		8		9		
Locus	012abc3456789012345					67890123456789012abc345678901234						
1-03	WINA--GNGNTKYSQKFQG					RVTITRDTSASTAYMELSSLRSED	TAVYYCAR					
"AT"	WINA GNGNTKFSQKFQG					RITVTRDTAASTAYMELRSLRSED	TAVYYCAR					
"Y"	WINA GNGNTKFSQKFQG					RITVTRDTAASTAYMELRSLRSED	TAVYYCAR					
CDR3												
-----												
Diversity												
3-10	VLLWFGELL*					YYYGSGSYYN		ITMVRGVII				
"AT"						DS		SNMVRGIIIAY				
"Y"						DS		SNMVRGIIIAY				
H3												
-----												
CDR3												
-----												
100		110										
Joining												
JH4	YFDYWGQGT					LVTVSS						
"AT"	YFDYWGQGT					LVTVSS						
"Y"	YFDYWGQGT					LVTVSS						

Figure 17

FAB "P" HEAVY CHAIN SEQUENCE COMPARISION TO GERMLINE

			H1		
			FR1	CDR1	FR2
			-----	-----	-----
	1	2	3		4
Locus	123456789012345678901234567890			1ab2345	67890123456789
3-30	QVQLVESGGGVVQPGRSLRLSCAASGFTFS			S--YGMH	WVRQAPGKGLEWVA
"P"	EVQLVQSGGGLVQPGGSLRLSCLVSGFTFN			N YPMH	WVRQAPGKGLEWVA

			H2		
			CDR2	FR3	
			-----	-----	-----
	5	6	7	8	9
Locus	012abc3456789012345		67890123456789012abc345678901234		
3-30	VISY--DGSNKYYADSVKG		RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK		
"P"	VISY DGNNKYYADSVKG		RFTISRDN SKNTLYLQMNSLRSEDTAVYYCAR		

Diversity Unknown  
 "P" GGG

			H3	
			CDR3	
			-----	
	100	110		
Joining				
JH4			YFDYWGQGTLVTVSS	
"P"			GFDYWGQGTLVTVSS	

Figure 18

FAB "S" HEAVY CHAIN SEQUENCE COMPARISON TO GERMLINE

H1									
-----									



Figure 19

FAB "AT" LIGHT CHAIN SEQUENCE COMPARISON TO GERMLINE

		L1		L2	
		FR1	CDR1	FR2	CDR2
		1 2	3	4	5
Locus		12345678901234567890123	45678901abcdef234	567890123456789	0123456
O12		DIQMTQSPSSLSASVGDRVTITC	RASQSISS-----YLN	WYQQKPGKAPKLLIY	AASSLQS
"AT"		EIVMTQSPSSLSASVGDRVTITC	RASQSISR YLN	WYQLKPGKAPRLLIY	GASSLQS
		L3			
		FR3	CDR3		
		6 7 8	9		
Locus		78901234567890123456789012345678	9012345		
O12		GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTP		
"AT"		GVPSRFSGSGSGAEFTLTISLQPEDIATYYC	QHTRA--		
		L3			
		CDR3			
		100			
Joining					
JK1		WTFGQGTKVEIK			
"AT"		--FGQGTKVEIK			

Figure 20

FAB "Y" LIGHT CHAIN SEQUENCE COMPARISON TO GERMLINE

CDR2	L1		L2
	FR1	CDR1	FR2
---	-----	-----	-----
	1 2	3	4 5
Locus	12345678901234567890123	45678901abcdef234	567890123456789
0123456			
L6	EIVLTQSPATLSLSPGERATLSC	RASQSVSS-----YLA	WYQKPGQAPRLLIY
DASNRAT			
"Y"	EIVLTQSPATLSFSPGERATLSC	RASQSVGS YLA	WYQQRPGQAPRPLIY
DATNRAT			
		L3	
	FR3	CDR3	
	-----	-----	
	6 7 8	9	
Locus	78901234567890123456789012345678	9012345	
L6	GIPARFSGSGSGTDFTLTISSELEPEDFAVYYC	QQRSNWP	
"Y"	GIPTRFSGSGSGTDFTLTISSELEPEDFATYYC	QHR----	
	L3		
	---		
	CDR3		
	---		
	100		
Joining			
JK2	YTFGQGTKLEIK		
"Y"	RTFGRGTKLEIK		

Figure 21

FAB "P" LIGHT CHAIN SEQUENCE COMPARISON TO GERMLINE

		L1		L2	
		FR1	CDR1	FR2	
CDR2					
		1 2	3	4	5
Locus	12345678901234567890123	45678901	abcdef234	567890123456789	
0123456					
A27	EIVLTQSPATLSLSPGERATLSC	RASQSVSS	-----YLA	WYQQKPGQAPRLLIY	
DASNRAT					
"P"	EIVMTQSPGTLSLSPGERATLSC	RASQSVSS	SSLA	WYQQKPGQAPRLLIY	
GASSRAT					
		L3			
		FR3	CDR3		
		6 7 8	9		
Locus	78901234567890123456789012345678	9012345			
A27	GIPARFSGSGSGTDFTLTISSELEPEDFAVYYC	QQRSNWP			
"P"	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQY----			
		L3			
		CDR3			
		100			
Joining					
JK4	LTFGGGTKVEIK				
"P"	GAFGGKTKVEIK				

Figure 22

FAB "S" LIGHT CHAIN SEQUENCE COMPARISON TO GERMLINE

	FR1		CDR1	FR2	CDR2
	1	2	3	4	5
Locus	1234567891234567890123	45678901abc234	567890123456789	01abcde23456	
*3m	SYELMQPPSVSVSPGQTARITC	SG-DA-LPKQ-YAY	WYQQKPGQAPVLVIY	KD-----SERPS	
"S"	QSVLTQPPSVSVSPGQTATITC	SG DA LPKQ YVY	WYRQKPGQAPLLVIY	ED SERPS	
	FR3		CDR3		
	6	7	8	9	
Locus	789012345678ab90123456789012345678	9012345abcde			
*3m	GIPERFSGSSSG--TTVTLTISGVQAEDEADYYC	QSDSSGTY			
"S"	GIPERFSGSSSG TEVTLSISGVQAEDEADYYC	QSTDSSGTY			
	CDR3				
	--				
	100				
Joining					
JL2	VVFGGGTKLTVL				
"S"	VVFGGGTKLTVL				

\* The variable region was identified but not yet named. The next closest match is "3m" shown above.

**Figure 23**  
**Raw Cell Bioassay**  
**"AT" 405, "AT" 406, "AT" 407**

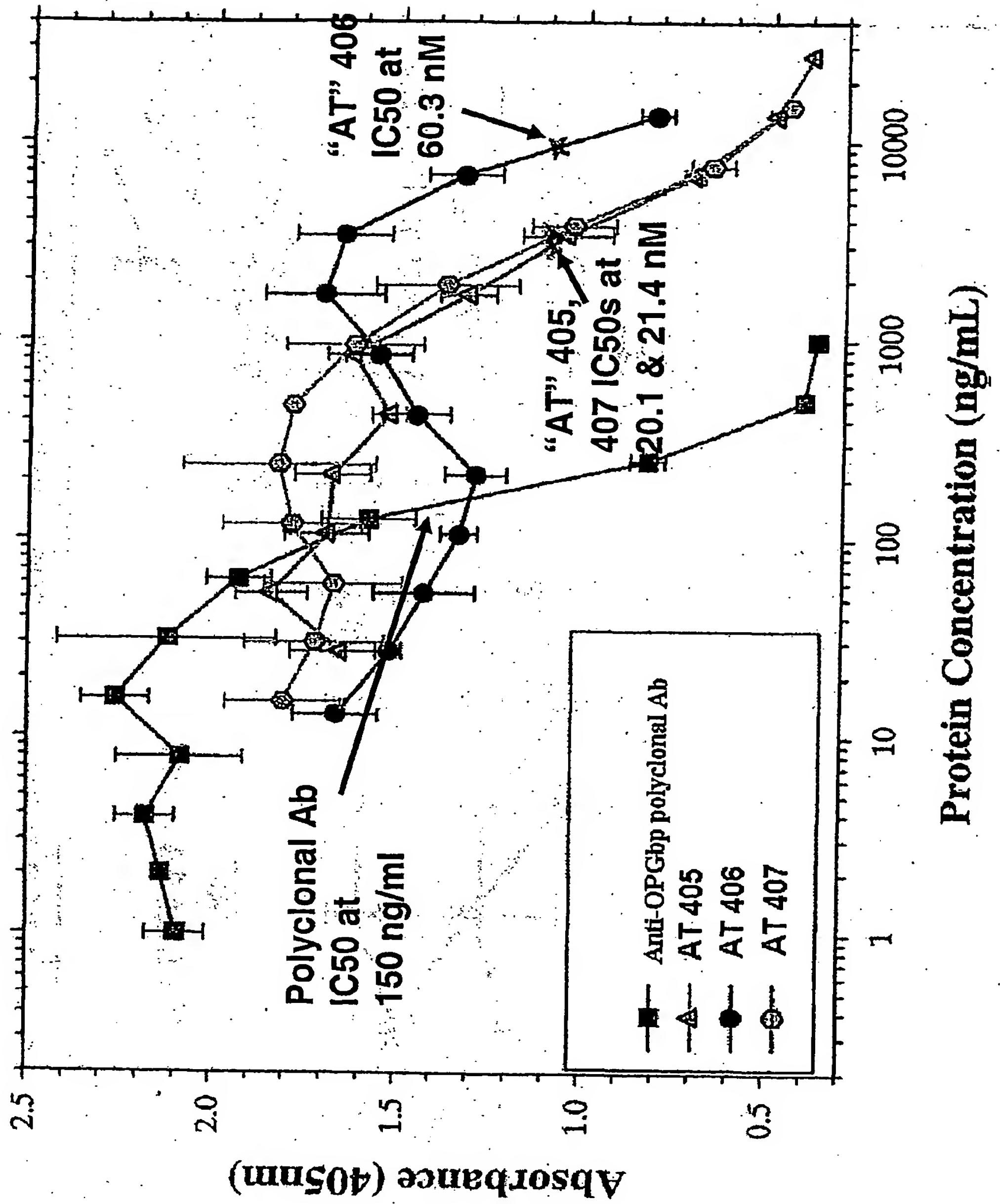
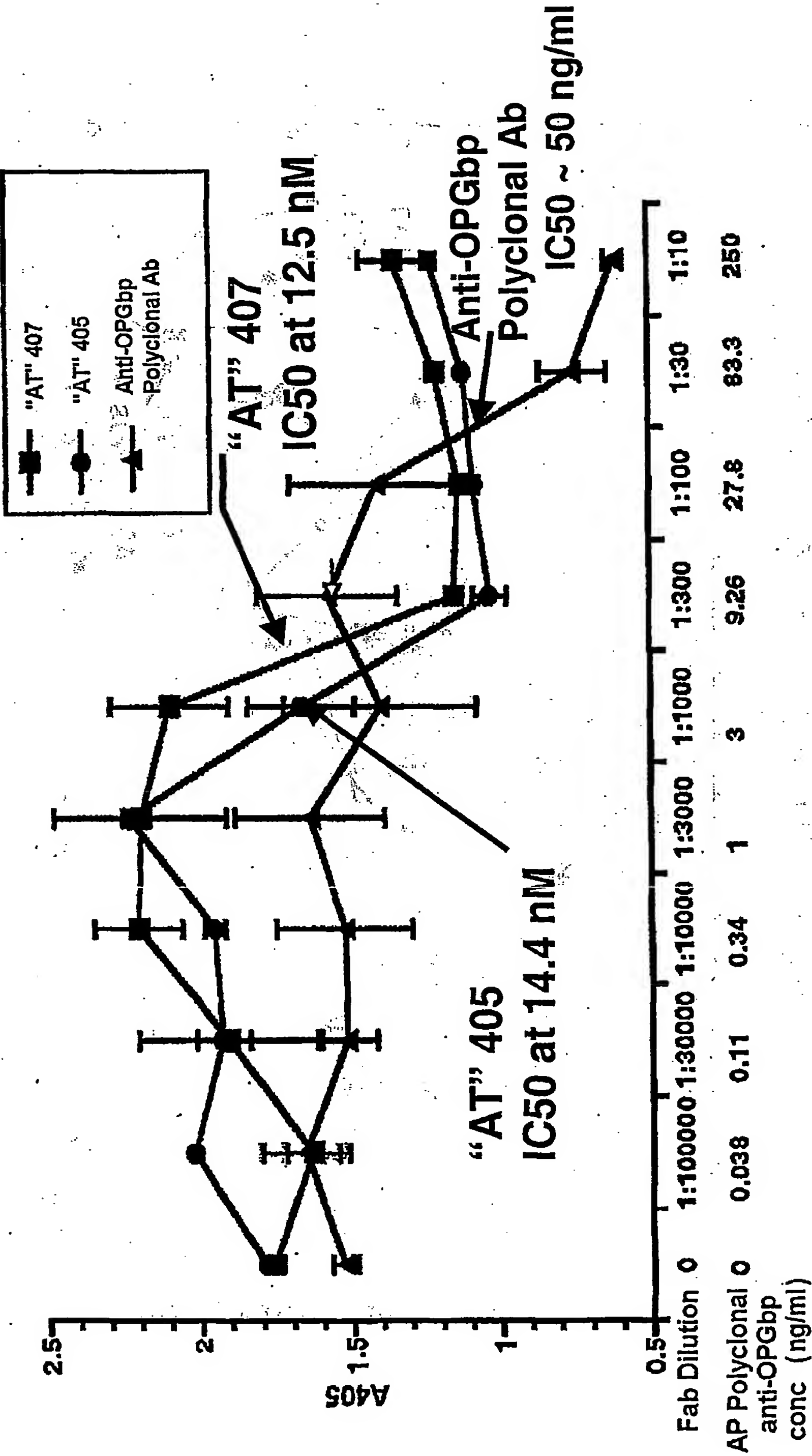
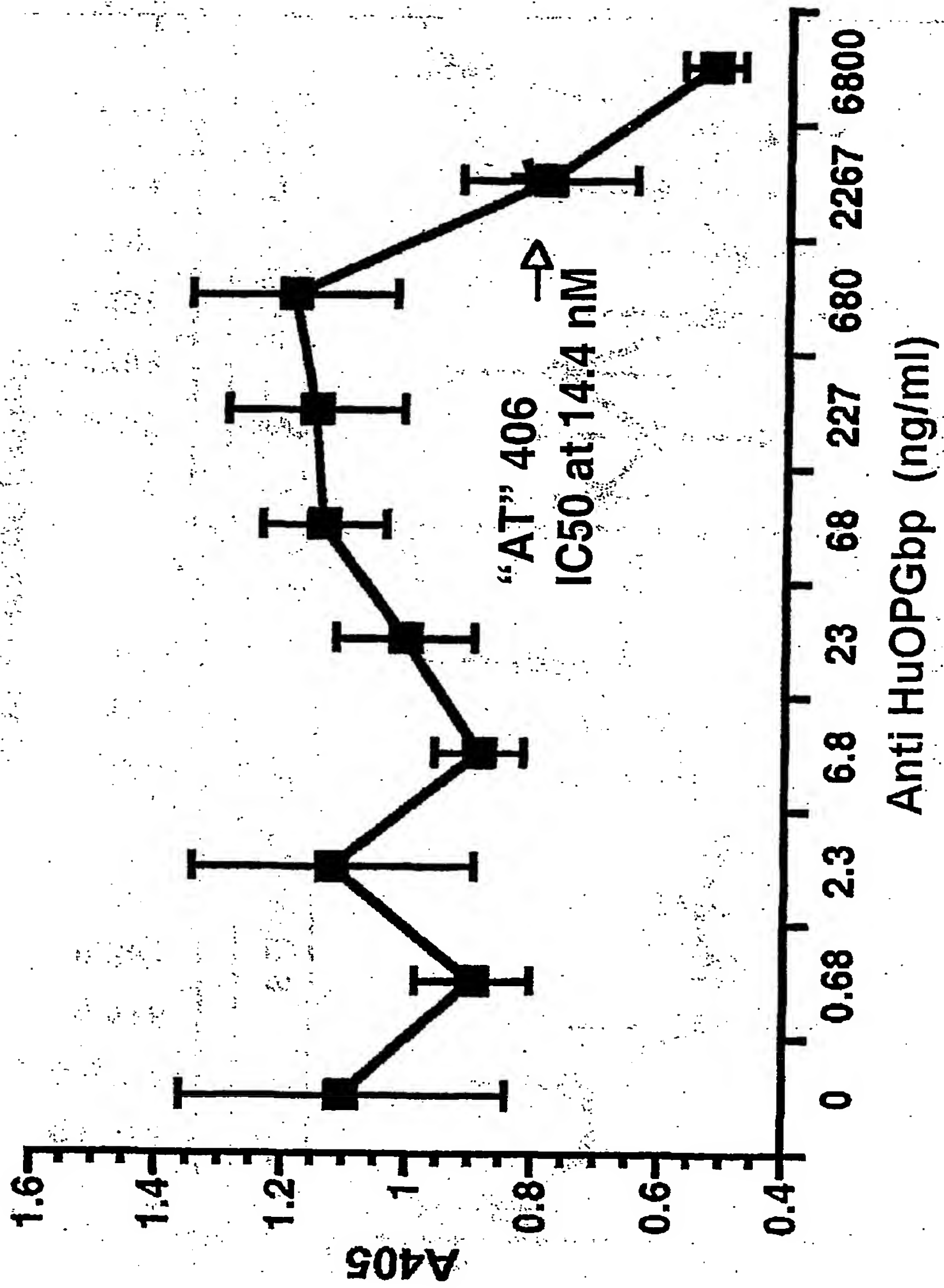


Figure 24  
Bone Marrow Assay  
"AT" 405, "AT" 407





**Figure 25**  
**Bone Marrow Assay "AT" 406**



**Figure 26**  
**Bone Marrow Assay**  
**"S" 435 and "Y" 429**

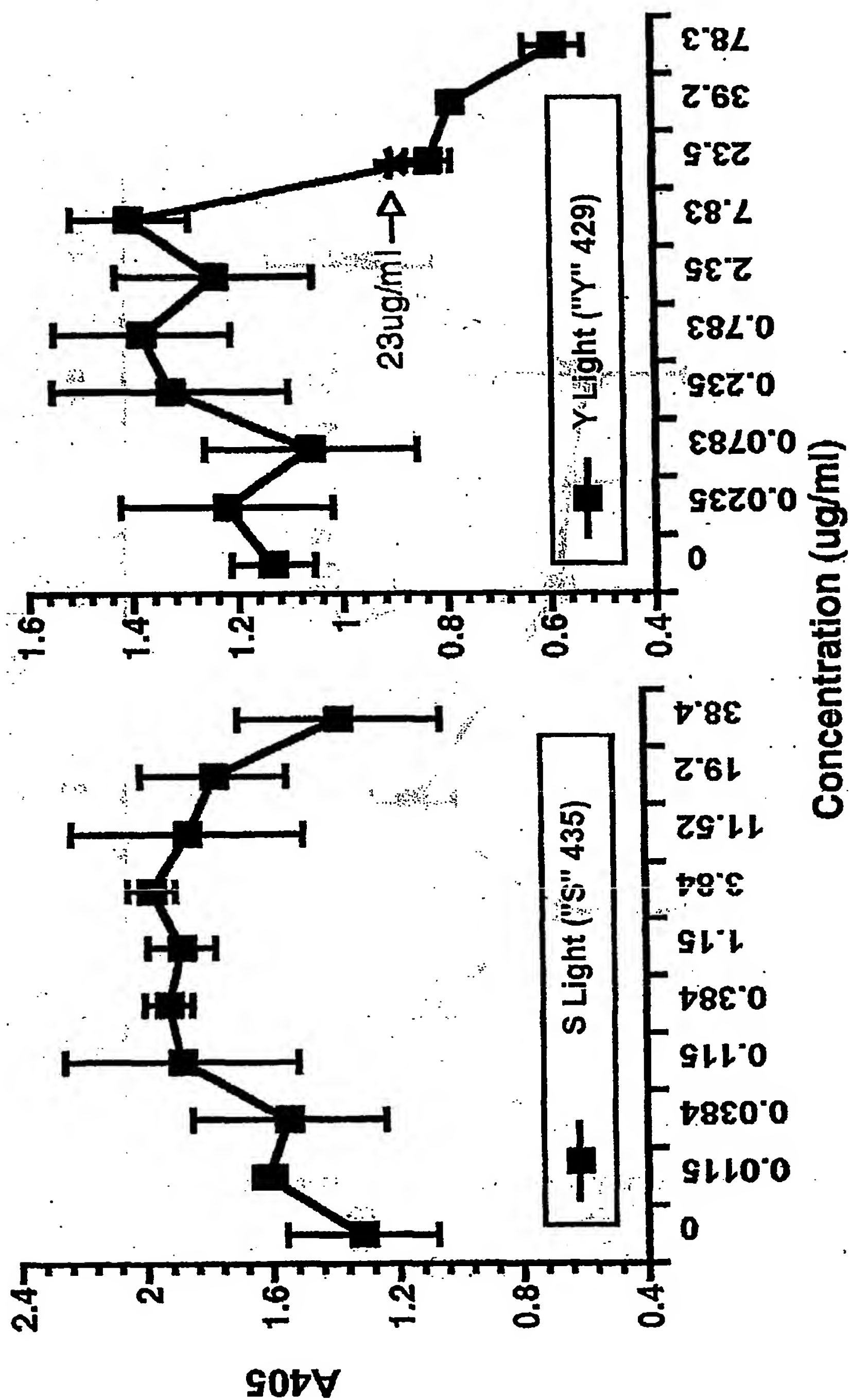
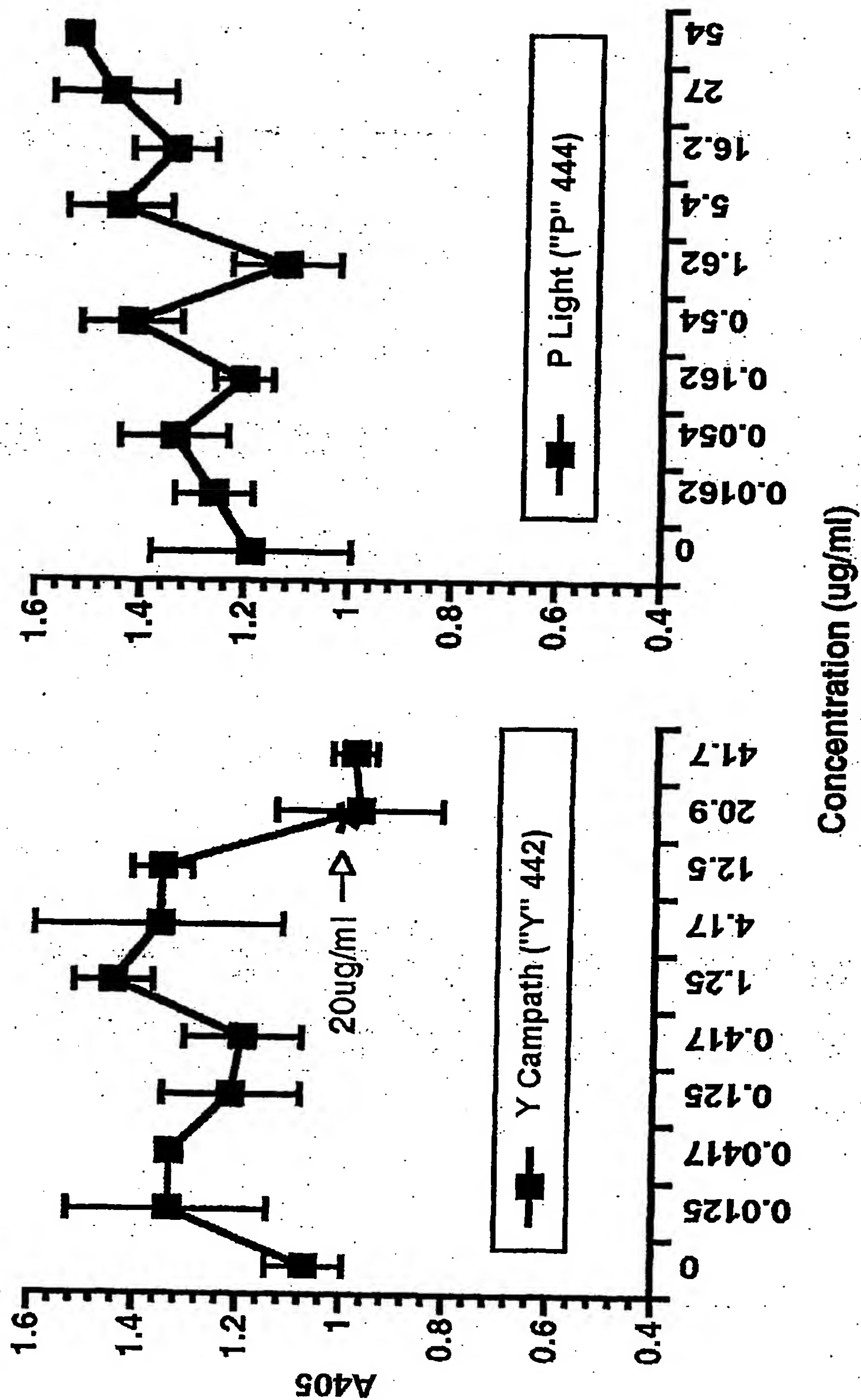


Figure 27  
Bone Marrow Assay  
"Y" 442 and "P" 444



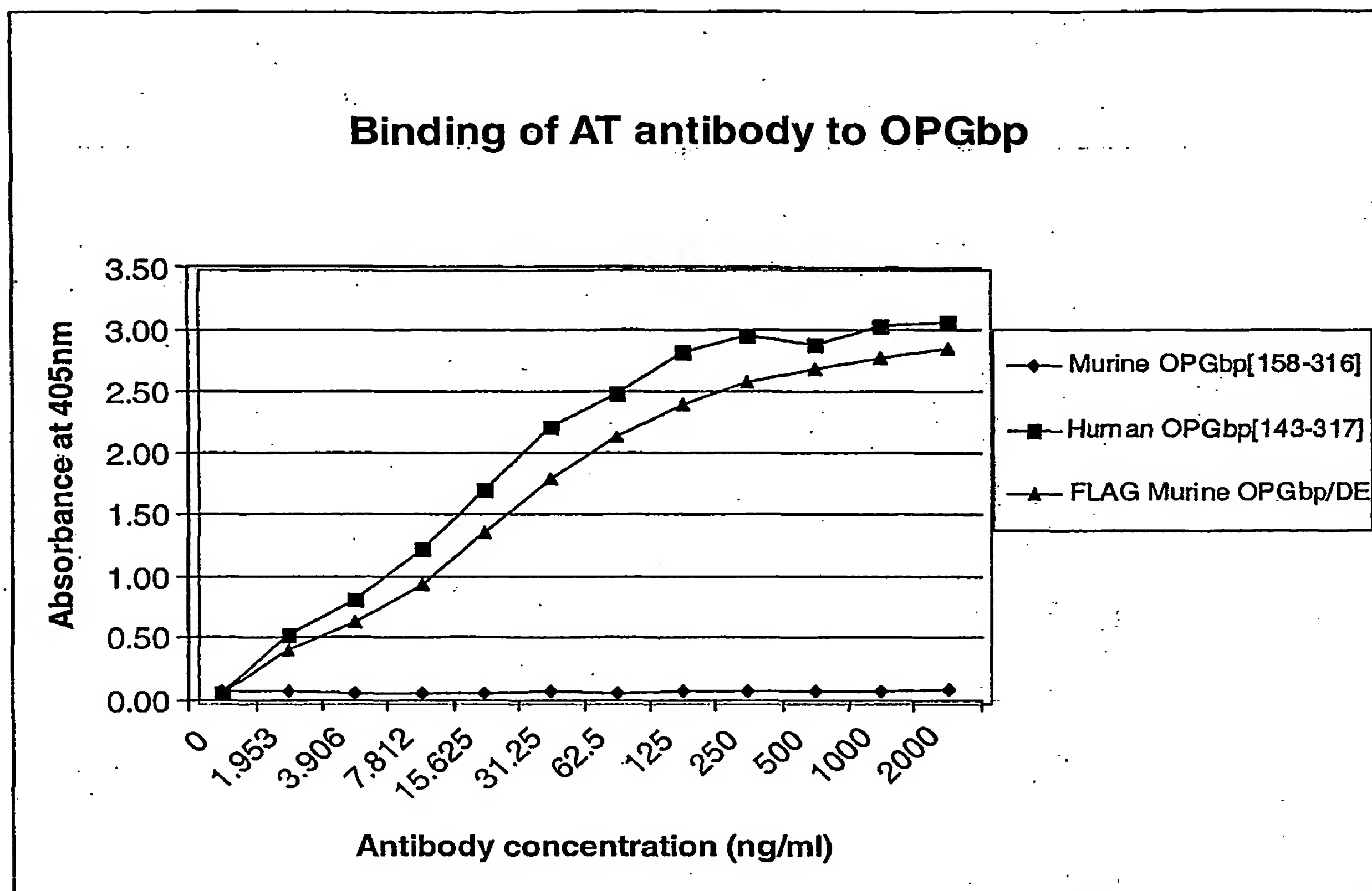
## FIGURE 28

cat	atg	gac	tac	aag	gac	gac	gat	gac	aag	aag	ctt	aag	cct	gag	gcc	48
Met	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Lys	Leu	Lys	Pro	Glu	Ala		
1				5				10						15		
cag	cca	ttt	gca	cac	ctc	acc	atc	aat	gct	gcc	agc	atc	cca	tcg	ggt	96
Gln	Pro	Phe	Ala	His	Leu	Thr	Ile	Asn	Ala	Ala	Ser	Ile	Pro	Ser	Gly	
			20					25					30			
tcc	cat	aaa	gtc	act	ctg	tcc	tct	tgg	tac	cac	gat	cga	ggc	tgg	gcc	144
Ser	His	Lys	Val	Thr	Leu	Ser	Ser	Trp	Tyr	His	Asp	Arg	Gly	Trp	Ala	
			35				40						45			
aag	atc	tct	aac	atg	acg	tta	agc	aac	gga	aaa	cta	agg	gtt	aac	caa	192
Lys	Ile	Ser	Asn	Met	Thr	Leu	Ser	Asn	Gly	Lys	Leu	Arg	Val	Asn	Gln	
		50					55				60					
gat	ggc	ttc	tat	tac	ctg	tac	gct	aac	att	tgc	ttt	cgg	cat	cat	gaa	240
Asp	Gly	Phe	Tyr	Tyr	Leu	Tyr	Ala	Asn	Ile	Cys	Phe	Arg	His	His	Glu	
	65				70					75						
aca	tcg	gga	gac	ctg	gct	act	gaa	tat	ctt	cag	ctg	atg	gtg	tat	gtc	288
Thr	Ser	Gly	Asp	Leu	Ala	Thr	Glu	Tyr	Leu	Gln	Leu	Met	Val	Tyr	Val	
80					85				90						95	
gtt	aaa	acc	agc	atc	aaa	atc	cca	agt	tct	cat	aac	ctg	atg	aaa	gga	336
Val	Lys	Thr	Ser	Ile	Lys	Ile	Pro	Ser	Ser	His	Asn	Leu	Met	Lys	Gly	
				100				105						110		
ggg	agc	acg	aaa	aac	tgg	tcg	ggc	aat	tct	gaa	ttc	cac	ttt	tat	tcc	384
Gly	Ser	Thr	Lys	Asn	Trp	Ser	Gly	Asn	Ser	Glu	Phe	His	Phe	Tyr	Ser	
			115					120					125			
ata	aat	gtt	ggg	gga	ttt	ttc	aag	ctc	cga	gct	ggt	gaa	gaa	att	agc	432
Ile	Asn	Val	Gly	Gly	Phe	Phe	Lys	Leu	Arg	Ala	Gly	Glu	Glu	Ile	Ser	
		130					135					140				
att	cag	gtg	tcc	aac	cct	tcc	ctg	ctg	gat	ccg	gat	caa	gat	gcg	acg	480
Ile	Gln	Val	Ser	Asn	Pro	Ser	Leu	Leu	Asp	Pro	Asp	Gln	Asp	Ala	Thr	
	145					150					155					
tac	ttt	ggg	gct	ttc	aaa	gtt	cag	gac	ata	gac	taa	ctc	gag			522
Tyr	Phe	Gly	Ala	Phe	Lys	Val	Gln	Asp	Ile	Asp						
160					165					170						

FIGURE 29

Human OPGbp [143-317] 212 GFYLYLANICFRHHETSGDLATEYLQLMVYVTKTSIKIP 250  
 |||||  
 Murine OPGbp [158-316] 211 GFYLYLANICFRHHETSGSVPTDYLQLMVYVVKTSIKIP 249  
 |||||  
 FLAG Murine OPGbp [158-316]/DE GFYLYLANICFRHHETSGDLATEYLQLMVYVVKTSIKIP

FIGURE 30





A-633A PCT.txt  
SEQUENCE LISTING

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&lt;120&gt; SELECTIVE BINDING AGENTS OF OSTEOPROTEGERIN BINDING PROTEIN

&lt;130&gt; A-633A

&lt;150&gt; 09/511,139

&lt;151&gt; 2000-02-23

&lt;160&gt; 154

&lt;170&gt; PatentIn version 3.0

&lt;210&gt; 1

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 1

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&lt;210&gt; 2

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 2

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&lt;210&gt; 3

&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 3

Arg Ala Ser Gln Ser Val Ser Ser Ser Ser Leu Ala  
1 5 10

&lt;210&gt; 4

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Human

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&lt;400&gt; 11

Gln Gln Tyr Gly Ala  
1 5

&lt;210&gt; 12

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 12

Gln Ser Thr Asp Ser Ser Gly Thr Tyr Val Val  
1 5 10

&lt;210&gt; 13

&lt;211&gt; 5

&lt;212&gt; PRT

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&lt;400&gt; 13

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1 5

&lt;210&gt; 14

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 14

Asn Tyr Pro Met His  
1 5

&lt;210&gt; 15

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 15

Asp Tyr Ala Met His  
1 5

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Gly

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1 5 10 15

Gly

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Tyr

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&lt;211&gt; 24

&lt;212&gt; DNA

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&lt;400&gt; 23

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&lt;210&gt; 24

&lt;211&gt; 20

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&lt;400&gt; 24

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&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Human

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A-633A PCT.txt

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Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Arg Gly Ala Arg

1

5

10

15

&lt;210&gt; 38

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 38

Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Arg Gly Ala Arg  
1 5 10 15

&lt;210&gt; 39

&lt;211&gt; 44

&lt;212&gt; DNA

&lt;213&gt; Human

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (2) .. (43)

&lt;400&gt; 39

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1

5

10

&lt;210&gt; 40

&lt;211&gt; 14

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 40

Trp Leu Arg Gly Ala Arg Cys Glu Ile Val Met Thr Gln Ser  
1 5 10

&lt;210&gt; 41

&lt;211&gt; 32

&lt;212&gt; DNA

&lt;213&gt; Human

## A-633A PCT.txt

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&lt;221&gt; CDS

&lt;222&gt; (20) .. (31)

&lt;400&gt; 41

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32

Thr Leu Ser Pro

1

&lt;210&gt; 42

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 42

Thr Leu Ser Pro

1

&lt;210&gt; 43

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 43

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
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20 25 30

&lt;210&gt; 44

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&lt;212&gt; DNA

&lt;213&gt; Human

&lt;220&gt;

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&lt;222&gt; (1) .. (645)

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5

10

15

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agc att agc aga tat tta aat tgg tat cag ctt aaa cca ggg aaa gcc  
 144  
 Ser Ile Ser Arg Tyr Leu Asn Trp Tyr Gln Leu Lys Pro Gly Lys Ala  
 35 40 45

cct agg ctc ctg atc tat ggt gca tcc agt ttg caa agt gga gtc cca  
 192  
 Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Leu Gln Ser Gly Val Pro  
 50 55 60

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 240  
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 65 70 75 80

agc agt cta caa cct gaa gac att gcc act tac tac tgt caa cac act  
 288  
 Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Thr  
 85 90 95

cgg gcg ttc ggc caa ggg acc aag gtt gaa atc aag cga act gtg gct  
 336  
 Arg Ala Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala  
 100 105 110

gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct  
 384  
 Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
 115 120 125

gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag  
 432  
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
 130 135 140

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gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc  
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Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser

145

150

155

160

cag gag agt gcc aca gag cag gac agc aag gac agc acc tac agc ctc  
528

Gln Glu Ser Ala Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu

165

170

175

agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc  
576

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val

180

185

190

tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca aag  
624

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys

195

200

205

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645

Ser Phe Asn Arg Gly Glu Cys

210

215

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20 25 30

Ser Ile Ser Arg Tyr Leu Asn Trp Tyr Gln Leu Lys Pro Gly Lys Ala  
35 40 45

## A-633A PCT.txt

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Leu Gln Ser Gly Val Pro  
50 55 60

Ser Arg Phe Ser Gly Ser Gly Ser Gly Ala Glu Phe Thr Leu Thr Ile  
65 70 75 80

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Thr  
85 90 95

Arg Ala Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala  
100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
145 150 155 160

Gln Glu Ser Ala Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys  
195 200 205

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210 215

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## A-633A PCT.txt

tct cac agt gca ctt gaa att gtg ctg act cag tct cca gcc acc ctg  
 48  
 Ser His Ser Ala Leu Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu

1 5 10 15

tct ttt tct ccg ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag  
 96  
 Ser Phe Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln

20 25 30

agt gtt ggc agc tac tta gcc tgg tac cag cag aga cct ggc cag gct  
 144  
 Ser Val Gly Ser Tyr Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln Ala

35 40 45

ccc agg ccc ctc atc tat gat gca acc aac agg gcc act ggc atc cca  
 192  
 Pro Arg Pro Leu Ile Tyr Asp Ala Thr Asn Arg Ala Thr Gly Ile Pro

50 55 60

acc agg ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc  
 240  
 Thr Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile

65 70 75 80

agc agc cta gag cct gaa gat ttt gca act tat tac tgt caa cac cga  
 288  
 Ser Ser Leu Glu Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Arg

85 90 95

agg act ttt ggc cgg ggg acc aag ttg gag atc aaa cga act gtg gct  
 336  
 Arg Thr Phe Gly Arg Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala

100 105 110

gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct  
 384  
 Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser

115 120 125

## A-633A PCT.txt

gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag  
 432  
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu

130

135

140

gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc  
 480  
 Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser

145

150

155

160

cag gag agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc  
 528  
 Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu

165

170

175

agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc  
 576  
 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val

180

185

190

tac gcc tgc gaa gtc act cat cag ggc ctg agc tcg ccc gtc aca aag  
 624  
 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys

195

200

205

agc ttc aac agg gga gag tgt  
 645  
 Ser Phe Asn Arg Gly Glu Cys

210

215

<210> 47  
 <211> 215  
 <212> PRT  
 <213> Human

<400> 47

Ser His Ser Ala Leu Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu  
 1 5 10 15

## A-633A PCT.txt

Ser Phe Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln  
 20 25 30  
 Ser Val Gly Ser Tyr Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln Ala  
 35 40 45  
 Pro Arg Pro Leu Ile Tyr Asp Ala Thr Asn Arg Ala Thr Gly Ile Pro  
 50 55 60  
 Thr Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 65 70 75 80  
 Ser Ser Leu Glu Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Arg  
 85 90 95  
 Arg Thr Phe Gly Arg Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala  
 100 105 110  
 Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
 115 120 125  
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
 130 135 140  
 Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
 145 150 155 160  
 Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
 165 170 175  
 Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
 180 185 190  
 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys  
 195 200 205  
 Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 48  
 <211> 645  
 <212> DNA

## A-633A PCT.txt

&lt;213&gt; Human

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(645)

&lt;400&gt; 48

cac agt gca ctt gaa att gtg atg aca cag tct cca ggc acc ctg tct

48

His Ser Ala Leu Glu Ile Val Met Thr Gln Ser Pro Gly Thr Leu Ser

1

5

10

15

ttg tct cca ggg gaa aga gcc acc ctc tcc tgc agg gcc agt cag agt

96

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser

20

25

30

gtt agc agc agc tcc tta gcc tgg tac cag cag aaa cct ggc cag gct

144

Val Ser Ser Ser Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala

35

40

45

ccc agg ctc ctc atc tat ggt gca tcc agc agg gcc act ggc atc cca

192

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro

50

55

60

gac agg ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc

240

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile

65

70

75

80

agc aga ctg gag cct gaa gat ttt gca gtg tat tac tgt cag cag tat

288

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr

85

90

95

ggt gct ttc ggc gga ggg acc aag gtg gag atc aaa cga act gtg gct

336

Gly Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala

100

105

110

## A-633A PCT.txt

gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct  
384

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser

115

120

125

gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag  
432

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu

130

135

140

gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc  
480

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser

145

150

155

160

cag gag agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc  
528

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu

165

170

175

agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc  
576

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val

180

185

190

tac gcc tgc gaa gtc acc cat cag ggc ctg aac tcg ccc gtc aca aag  
624

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Asn Ser Pro Val Thr Lys

195

200

205

agc ttc aac agg gga gag tgt  
645

Ser Phe Asn Arg Gly Glu Cys

210

215

<210> 49  
<211> 215  
<212> PRT  
<213> Human

## A-633A PCT.txt

&lt;400&gt; 49

His Ser Ala Leu Glu Ile Val Met Thr Gln Ser Pro Gly Thr Leu Ser  
 1 5 10 15

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser  
 20 25 30

Val Ser Ser Ser Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala  
 35 40 45

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro  
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
 65 70 75 80

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr  
 85 90 95

Gly Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala  
 100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser  
 115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu  
 130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser  
 145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu  
 165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
 180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Asn Ser Pro Val Thr Lys  
 195 200 205

## A-633A PCT.txt

Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 50  
 <211> 654  
 <212> DNA  
 <213> Human

<220>  
 <221> CDS  
 <222> (1) .. (654)

<400> 50  
 tct cac agt gca cag tct gtg ctg act cag cca ccc tcg gtg tca gtg  
 48  
 Ser His Ser Ala Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val  
 1 5 10 15

tcc cca gga cag acg gcc acg atc acc tgc tct gga gat gca ttg cca  
 96  
 Ser Pro Gly Gln Thr Ala Thr Ile Thr Cys Ser Gly Asp Ala Leu Pro  
 20 25 30

aag caa tat gtt tat tgg tac cgg cag aag cca ggc cag gcc cct cta  
 144  
 Lys Gln Tyr Val Tyr Trp Tyr Arg Gln Lys Pro Gly Gln Ala Pro Leu  
 35 40 45

ttg gtg ata tat gaa gac agt gag agg ccc tca ggg atc cct gaa cga  
 192  
 Leu Val Ile Tyr Glu Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg  
 50 55 60

ttc tct ggc tcc agt tca ggg act gaa gtc acg ttg agt atc agt gga  
 240  
 Phe Ser Gly Ser Ser Ser Gly Thr Glu Val Thr Leu Ser Ile Ser Gly  
 65 70 75 80

gtc cag gca gaa gac gag gct gac tat tat tgt caa tca aca gac agc  
 288  
 Val Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Thr Asp Ser  
 85 90 95



## A-633A PCT.txt

agt ggg act tat gtc gtc ttc ggc gga ggg acc aag ctg acc gtc cta  
336

Ser Gly Thr Tyr Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu

100

105

110

agt cag ccc aag gct gcc ccc tcg gtc act ctg ttc ccg ccc tcc tct  
384

Ser Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser

115

120

125

gag gag ctt caa gcc aac aag gcc aca ctg gtg tgt ctc ata agt gac  
432

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp

130

135

140

ttc tac ccg gga gcc gtg aca gtg gcc tgg aag gca gat agc agc ccc  
480

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro

145

150

155

160

gtc aag gcg gga gtg gag acc acc aca ccc tcc aaa caa agc aac aac  
528

Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn

165

170

175

aag tac gcg gcc agc agc tat ctg agc ctg acg cct gag cag tgg aag  
576

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys

180

185

190

tcc cac aga agc tac agc tgc cag gtc acg cat gaa ggg agc acc gtg  
624

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val

195

200

205

gag aag aca gtg gcc cct aca gaa tgt tca  
654

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser

210

215

## A-633A PCT.txt

<210> 51  
 <211> 218  
 <212> PRT  
 <213> Human

<400> 51

Ser His Ser Ala Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val  
 1 5 10 15

Ser Pro Gly Gln Thr Ala Thr Ile Thr Cys Ser Gly Asp Ala Leu Pro  
 20 25 30

Lys Gln Tyr Val Tyr Trp Tyr Arg Gln Lys Pro Gly Gln Ala Pro Leu  
 35 40 45

Leu Val Ile Tyr Glu Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg  
 50 55 60

Phe Ser Gly Ser Ser Ser Gly Thr Glu Val Thr Leu Ser Ile Ser Gly  
 65 70 75 80

Val Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Thr Asp Ser  
 85 90 95

Ser Gly Thr Tyr Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
 100 105 110

Ser Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser  
 115 120 125

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp  
 130 135 140

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro  
 145 150 155 160

Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn  
 165 170 175

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys

A-633A PCT.txt

180

185

190

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val  
 195 200 205

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser  
 210 215

<210> 52  
 <211> 690  
 <212> DNA  
 <213> Human

<220>  
 <221> CDS  
 <222> (1) .. (690)

<400> 52

gcc cag gtc cag ctg gtg cag tct ggg gct gag gtg agg aag cct ggg  
 48

Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly

1 5 10 15

gcc tca gtg aag gtt tcc tgc aag gct tct gga tac gac ttc agt aat  
 96

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Asp Phe Ser Asn

20 25 30

tat gct ata cat tgg gtg cgc cag gcc ccc gga caa agg ctt gag tgg  
 144

Tyr Ala Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp

35 40 45

atg gga tgg atc aac gct ggc aat ggg aac aca aaa ttt tca cag aag  
 192

Met Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Phe Ser Gln Lys

50 55 60

ttc cag ggc aga atc acc gtt acc agg gac aca gcc gcg agc aca gcc  
 240

Phe Gln Gly Arg Ile Thr Val Thr Arg Asp Thr Ala Ala Ser Thr Ala

65 70 75 80

## A-633A PCT.txt

tac atg gag ctg cgc agt ctg aga tct gaa gac acg gct gtg tat tac  
288

Tyr Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr

85

90

95

tgt gcg aga gat tcc tca aat atg gtt cgg gga att att ata gcg tac  
336

Cys Ala Arg Asp Ser Ser Asn Met Val Arg Gly Ile Ile Ile Ala Tyr

100

105

110

tat ttt gac tac tgg ggc cag ggc acc ctg gtc acc gtc tca agc gcc  
384

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala

115

120

125

tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc  
432

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser

130

135

140

acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc  
480

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe

145

150

155

160

ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc  
528

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly

165

170

175

gtc cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc  
576

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu

180

185

190

agc agc gta gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac  
624

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr

195

200

205

## A-633A PCT.txt

atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa  
672

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys

210

215

220

gtt gag ccc aaa tct tgt

690

Val Glu Pro Lys Ser Cys

225

230

<210> 53  
<211> 230  
<212> PRT  
<213> Human

<400> 53

Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly  
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Asp Phe Ser Asn  
20 25 30

Tyr Ala Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp  
35 40 45

Met Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Phe Ser Gln Lys  
50 55 60

Phe Gln Gly Arg Ile Thr Val Thr Arg Asp Thr Ala Ala Ser Thr Ala  
65 70 75 80

Tyr Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr  
85 90 95

Cys Ala Arg Asp Ser Ser Asn Met Val Arg Gly Ile Ile Ile Ala Tyr  
100 105 110

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala  
115 120 125

## A-633A PCT.txt

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser  
 130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe  
 145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
 165 170 175

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
 180 185 190

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr  
 195 200 205

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys  
 210 215 220

Val Glu Pro Lys Ser Cys  
 225 230

<210> 54  
 <211> 690  
 <212> DNA  
 <213> Human

<220>  
 <221> CDS  
 <222> (1) .. (690)

<400> 54  
 gcc gag gtc cag ctg gtg cag tct ggg gct gag gtg agg aag cct ggg  
 48  
 Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly  
 1 5 10 15

gcc tca gtg aag gtt tcc tgc aag gct tct gga tac gac ttc agt aat  
 96  
 Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Asp Phe Ser Asn  
 20 25 30

tat gct ata cat tgg gtg cgc cag gcc ccc gga caa agg ctt gag tgg  
 144

## A-633A PCT.txt

Tyr Ala Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp

35

40

45

atg gga tgg atc aac gct ggc aat ggg aac aca aaa ttt tca cag aag

192

Met Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Phe Ser Gln Lys

50

55

60

ttc cag ggc aga atc acc gtt acc agg gac aca gcc gcg agc aca gcc

240

Phe Gln Gly Arg Ile Thr Val Thr Arg Asp Thr Ala Ala Ser Thr Ala

65

70

75

80

tac atg gag ctg cgc agt ctg aga tct gaa gac acg gct gtg tat tac

288

Tyr Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr

85

90

95

tgt gcg aga gat tcc tca aat atg gtt cgg gga att att ata gcg tac

336

Cys Ala Arg Asp Ser Ser Asn Met Val Arg Gly Ile Ile Ile Ala Tyr

100

105

110

tat ttt gac tac tgg ggc cag ggc acc ctg gtc acc gtc tca agc gcc

384

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala

115

120

125

tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc

432

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser

130

135

140

acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc

480

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe

145

150

155

160

ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc



## A-633A PCT.txt

528

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly

165

170

175

gtc cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc

576

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu

180

185

190

agc agc gta gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac

624

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr

195

200

205

atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa

672

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys

210

215

220

gtt gag ccc aaa tct tgt

690

Val Glu Pro Lys Ser Cys

225

230

&lt;210&gt; 55

&lt;211&gt; 230

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 55

Ala Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly

1

5

10

15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Asp Phe Ser Asn

20

25

30

Tyr Ala Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp

35

40

45

Met Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Phe Ser Gln Lys

## A-633A PCT.txt

50

55

60

Phe Gln Gly Arg Ile Thr Val Thr Arg Asp Thr Ala Ala Ser Thr Ala  
65 70 75 80

Tyr Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr  
85 90 95

Cys Ala Arg Asp Ser Ser Asn Met Val Arg Gly Ile Ile Ile Ala Tyr  
100 105 110

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala  
115 120 125

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser  
130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe  
145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
165 170 175

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
180 185 190

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr  
195 200 205

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys  
210 215 220

Val Glu Pro Lys Ser Cys  
225 230

<210> 56  
<211> 660  
<212> DNA  
<213> Human

<220>  
<221> CDS  
<222> (1)..(660)

## A-633A PCT.txt

&lt;400&gt; 56

gcc gag gtc cag ctg gtg cag tct ggg gga ggc ttg gtc cag cct ggg

48

Ala Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly

1

5

10

15

ggg tcc ctg aga ctc tcc tgt tta gtc tct gga ttc acc ttc aat aac

96

Gly Ser Leu Arg Leu Ser Cys Leu Val Ser Gly Phe Thr Phe Asn Asn

20

25

30

tat cct atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg

144

Tyr Pro Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp

35

40

45

gtg gca gtt ata tca tat gat gga aat aat aaa tac tac gca gac tcc

192

Val Ala Val Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser

50

55

60

gtg aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg

240

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

65

70

75

80

tat ttg caa atg aac agc ctg aga tct gag gac acg gcc gtg tat tac

288

Tyr Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr

85

90

95

tgt gcg agg ggg ggc ggt ggc ttt gac tac tgg ggc cag gga acc ctg

336

Cys Ala Arg Gly Gly Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu

100

105

110

gtc acc gtc tca agc gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg

384

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu

A-633A PCT.txt

115

120

125

gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc  
432

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys

130

135

140

ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca  
480

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser

145

150

155

160

ggc gcc ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tcc  
528

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser

165

170

175

tca gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc agc  
576

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser

180

185

190

ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac  
624

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn

195

200

205

acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt  
660

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys

210

215

220

<210> 57  
<211> 220  
<212> PRT  
<213> Human

<400> 57

Ala Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly  
1 5 10 15

## A-633A PCT.txt

Gly Ser Leu Arg Leu Ser Cys Leu Val Ser Gly Phe Thr Phe Asn Asn  
20 25 30

Tyr Pro Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp  
35 40 45

Val Ala Val Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser  
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu  
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr  
85 90 95

Cys Ala Arg Gly Gly Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu  
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu  
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys  
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser  
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser  
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser  
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn  
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
210 215 220

<210> 58

## A-633A PCT.txt

<211> 681  
 <212> DNA  
 <213> Human

<220>  
 <221> CDS  
 <222> (1)..(681)

<400> 58

gcc gag gtg cag ctg ctg gag tct ggg gga ggc ttg gta caa cct ggc  
 48

Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

1 5 10 15

agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttt gat gat  
 96

Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp

20 25 30

tat gcc atg cac tgg gtc cgg caa gct cca ggg aag ggc ctg gag tgg  
 144

Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp

35 40 45

gtc tca ggt att agt tgg aat agt ggt agg ata ggc tat gcg gac tct  
 192

Val Ser Gly Ile Ser Trp Asn Ser Gly Arg Ile Gly Tyr Ala Asp Ser

50 55 60

gtg aag ggc cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg  
 240

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu

65 70 75 80

tat ctg caa atg aac agt ctg aga cct gag gac acg gcc ttc tat tac  
 288

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Phe Tyr Tyr

85 90 95

tgt gca aaa ggg ggt tct aca agc gcg agg tat agc agt ggc tgg tac  
 336

Cys Ala Lys Gly Gly Ser Thr Ser Ala Arg Tyr Ser Ser Gly Trp Tyr

## A-633A PCT.txt

100

105

110

tac tgg ggc cag ggc acc ctg gtc acc gtc tca agc gcc tcc acc aag

384

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys

115

120

125

ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg

432

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly

130

135

140

ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg

480

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro

145

150

155

160

gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtc cac acc

528

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr

165

170

175

ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gta

576

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val

180

185

190

gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac

624

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn

195

200

205

gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc

672

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro

210

215

220

aaa tct tgt

681

Lys Ser Cys



## A-633A PCT.txt

225

<210> 59  
 <211> 227  
 <212> PRT  
 <213> Human

<400> 59

Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly  
 1 5 10 15

Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp  
 20 25 30

Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp  
 35 40 45

Val Ser Gly Ile Ser Trp Asn Ser Gly Arg Ile Gly Tyr Ala Asp Ser  
 50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu  
 65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Phe Tyr Tyr  
 85 90 95

Cys Ala Lys Gly Gly Ser Thr Ser Ala Arg Tyr Ser Ser Gly Trp Tyr  
 100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 165 170 175

## A-633A PCT.txt

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
                   180                  185                  190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
           195                  200                  205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
       210                  215                  220

Lys Ser Cys  
 225

<210> 60  
 <211> 141  
 <212> PRT  
 <213> Human

<400> 60

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1                  5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
           20                  25                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met  
           35                  40                  45

Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Tyr Ser Gln Lys Phe  
       50                  55                  60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr  
 65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
           85                  90                  95

Ala Arg Val Leu Leu Trp Phe Gly Glu Leu Leu Tyr Tyr Tyr Gly Ser  
           100                  105                  110

Gly Ser Tyr Tyr Asn Ile Thr Met Val Arg Gly Val Ile Ile Tyr Phe  
           115                  120                  125

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
       130                  135                  140

<210> 61  
 <211> 126  
 <212> PRT  
 <213> Human

## A-633A PCT.txt

&lt;400&gt; 61

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Asp Phe Ser Asn Tyr  
 20 25 30  
 Ala Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met  
 35 40 45  
 Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Phe Ser Gln Lys Phe  
 50 55 60  
 Gln Gly Arg Ile Thr Val Thr Arg Asp Thr Ala Ala Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Asp Ser Ser Asn Met Val Arg Gly Ile Ile Ile Ala Tyr Tyr  
 100 105 110  
 Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120 125

&lt;210&gt; 62

&lt;211&gt; 126

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 62

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Arg Lys Pro Gly Ala  
 1 5 10 15  
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Asp Phe Ser Asn Tyr  
 20 25 30  
 Ala Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met  
 35 40 45  
 Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Phe Ser Gln Lys Phe  
 50 55 60  
 Gln Gly Arg Ile Thr Val Thr Arg Asp Thr Ala Ala Ser Thr Ala Tyr  
 65 70 75 80  
 Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Arg Asp Ser Ser Asn Met Val Arg Gly Ile Ile Ile Ala Tyr Tyr  
 100 105 110

## A-633A PCT.txt

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120 125

<210> 63  
 <211> 113  
 <212> PRT  
 <213> Human

<400> 63

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Ala Lys Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser  
 100 105 110

Ser

<210> 64  
 <211> 116  
 <212> PRT  
 <213> Human

<400> 64

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Leu Val Ser Gly Phe Thr Phe Asn Asn Tyr  
 20 25 30  
 Pro Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ala Val Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80

## A-633A PCT.txt

Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
                   85                                  90                                  95

Ala Arg Gly Gly Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
                   100                                  105                                  110

Thr Val Ser Ser  
                   115

<210> 65  
 <211> 132  
 <212> PRT  
 <213> Human

<400> 65

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg  
 1                  5                                  10                                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr  
                   20                                  25                                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
                   35                                  40                                  45

Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val  
                   50                                  55                                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
 65                                  70                                  75                                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys  
                   85                                  90                                  95

Ala Lys Asp Gly Tyr Ser Ser Gly Trp Tyr Gly Ile Ala Val Ala Gly  
                   100                                  105                                  110

Val Gln Trp Leu Val Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val  
                   115                                  120                                  125

Thr Val Ser Ser  
                   130

<210> 66  
 <211> 123  
 <212> PRT  
 <213> Human

<400> 66

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg  
 1                  5                                  10                                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr

## A-633A PCT.txt

20

25

30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Arg Ile Gly Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Phe Tyr Tyr Cys  
85 90 95

Ala Lys Gly Gly Ser Thr Ser Ala Arg Tyr Ser Ser Gly Trp Tyr Tyr  
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 67  
<211> 107  
<212> PRT  
<213> Human

<400> 67

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Trp  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 68  
<211> 103  
<212> PRT  
<213> Human

<400> 68

## A-633A PCT.txt

Glu Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Arg Tyr  
20 25 30

Leu Asn Trp Tyr Gln Leu Lys Pro Gly Lys Ala Pro Arg Leu Leu Ile  
35 40 45

Tyr Gly Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Ala Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Thr Arg Ala Phe Gly Gln  
85 90 95

Gly Thr Lys Val Glu Ile Lys  
100

<210> 69  
<211> 107  
<212> PRT  
<213> Human

<400> 69

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr  
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile  
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Tyr  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 70  
<211> 103  
<212> PRT  
<213> Human

<400> 70



## A-633A PCT.txt

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Phe Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Gly Ser Tyr  
 20 25 30  
 Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Arg Pro Leu Ile  
 35 40 45  
 Tyr Asp Ala Thr Asn Arg Ala Thr Gly Ile Pro Thr Arg Phe Ser Gly  
 50 55 60  
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
 65 70 75 80  
 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Arg Arg Thr Phe Gly Arg  
 85 90 95  
 Gly Thr Lys Leu Glu Ile Lys  
 100

<210> 71  
 <211> 107  
 <212> PRT  
 <213> Human

<400> 71

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr  
 20 25 30  
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile  
 35 40 45  
 Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly  
 50 55 60  
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
 65 70 75 80  
 Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Leu  
 85 90 95  
 Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 72  
 <211> 104  
 <212> PRT  
 <213> Human

## A-633A PCT.txt

&lt;400&gt; 72

Glu Ile Val Met Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly  
 1 5 10 15  
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser  
 20 25 30  
 Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu  
 35 40 45  
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu  
 65 70 75 80  
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ala Phe Gly  
 85 90 95  
 Gly Gly Thr Lys Val Glu Ile Lys  
 100

<210> 73  
 <211> 108  
 <212> PRT  
 <213> Human

&lt;400&gt; 73

Ser Tyr Glu Leu Met Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln  
 1 5 10 15  
 Thr Ala Arg Ile Thr Cys Ser Gly Asp Ala Leu Pro Lys Gln Tyr Ala  
 20 25 30  
 Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr  
 35 40 45  
 Lys Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser  
 50 55 60  
 Ser Ser Gly Thr Thr Val Thr Leu Thr Ile Ser Gly Val Gln Ala Glu  
 65 70 75 80  
 Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ala Asp Ser Ser Gly Thr Tyr  
 85 90 95  
 Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
 100 105

<210> 74  
 <211> 108  
 <212> PRT  
 <213> Human

## A-633A PCT.txt

&lt;400&gt; 74

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln  
 1 5 10 15  
 Thr Ala Thr Ile Thr Cys Ser Gly Asp Ala Leu Pro Lys Gln Tyr Val  
 20 25 30  
 Tyr Trp Tyr Arg Gln Lys Pro Gly Gln Ala Pro Leu Leu Val Ile Tyr  
 35 40 45  
 Glu Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser  
 50 55 60  
 Ser Ser Gly Thr Glu Val Thr Leu Ser Ile Ser Gly Val Gln Ala Glu  
 65 70 75 80  
 Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Thr Asp Ser Ser Gly Thr Tyr  
 85 90 95  
 Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu  
 100 105

<210> 75  
 <211> 522  
 <212> DNA  
 <213> Murine

<220>  
 <221> CDS  
 <222> (4)..(513)

&lt;400&gt; 75

cat atg gac tac aag gac gac gat gac aag aag ctt aag cct gag gcc  
 48  
 Met Asp Tyr Lys Asp Asp Asp Asp Lys Lys Leu Lys Pro Glu Ala  
 1 5 10 15  
 cag cca ttt gca cac ctc acc atc aat gct gcc agc atc cca tcg ggt  
 96  
 Gln Pro Phe Ala His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly  
 20 25 30  
 tcc cat aaa gtc act ctg tcc tct tgg tac cac gat cga ggc tgg gcc  
 144  
 Ser His Lys Val Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala  
 35 40 45

## A-633A PCT.txt

aag atc tct aac atg acg tta agc aac gga aaa cta agg gtt aac caa  
 192  
 Lys Ile Ser Asn Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln  
 50 55 60

gat ggc ttc tat tac ctg tac gct aac att tgc ttt cgg cat cat gaa  
 240  
 Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu  
 65 70 75

aca tcg gga gac ctg gct act gaa tat ctt cag ctg atg gtg tat gtc  
 288  
 Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val Tyr Val  
 80 85 90 95

gtt aaa acc agc atc aaa atc cca agt tct cat aac ctg atg aaa gga  
 336  
 Val Lys Thr Ser Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly  
 100 105 110

ggg agc acg aaa aac tgg tcg ggc aat tct gaa ttc cac ttt tat tcc  
 384  
 Gly Ser Thr Lys Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser  
 115 120 125

ata aat gtt ggg gga ttt ttc aag ctc cga gct ggt gaa gaa att agc  
 432  
 Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser  
 130 135 140

att cag gtg tcc aac cct tcc ctg ctg gat ccg gat caa gat gcg acg  
 480  
 Ile Gln Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr  
 145 150 155

tac ttt ggg gct ttc aaa gtt cag gac ata gac taactcgag  
 522  
 Tyr Phe Gly Ala Phe Lys Val Gln Asp Ile Asp  
 160 165 170

## A-633A PCT.txt

<210> 76  
 <211> 170  
 <212> PRT  
 <213> Murine  
 <400> 76

Met Asp Tyr Lys Asp Asp Asp Asp Lys Lys Leu Lys Pro Glu Ala Gln  
 1 5 10 15

Pro Phe Ala His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser  
 20 25 30

His Lys Val Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys  
 35 40 45

Ile Ser Asn Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp  
 50 55 60

Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr  
 65 70 75 80

Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val Tyr Val Val  
 85 90 95

Lys Thr Ser Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly  
 100 105 110

Ser Thr Lys Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile  
 115 120 125

Asn Val Gly Gly Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile  
 130 135 140

Gln Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr  
 145 150 155 160

Phe Gly Ala Phe Lys Val Gln Asp Ile Asp  
 165 170

<210> 77  
 <211> 39

## A-633A PCT.txt

<212> PRT  
 <213> Human

<400> 77

Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr  
 1 5 10 15

Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val Tyr Val Thr  
 20 25 30

Lys Thr Ser Ile Lys Ile Pro  
 35

<210> 78  
 <211> 39  
 <212> PRT  
 <213> Murine

<400> 78

Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr  
 1 5 10 15

Ser Gly Ser Val Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val  
 20 25 30

Lys Thr Ser Ile Lys Ile Pro  
 35

<210> 79  
 <211> 39  
 <212> PRT  
 <213> Murine

<400> 79

Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr  
 1 5 10 15

Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val Tyr Val Val  
 20 25 30

Lys Thr Ser Ile Lys Ile Pro  
 35

<210> 80  
 <211> 17  
 <212> PRT  
 <213> Synthetic

<220>

<221> misc\_feature

<223> X is any amino acid different from the amino acid normally r  
 eside

A-633A PCT.txt

nt at that positio

&lt;400&gt; 80

Xaa	Ser	Ser	Asn	Met	Val	Arg	Gly	Ile	Ile	Ile	Ala	Tyr	Tyr	Phe	Asp
1				5					10					15	

Tyr

&lt;210&gt; 81

&lt;211&gt; 17

&lt;212&gt; PRT

&lt;213&gt; Synthetic

&lt;220&gt;

&lt;221&gt; misc\_feature

<223> X is any amino acid different from the amino acid normally r  
eside

nt at that positio

&lt;400&gt; 81

Asp	Xaa	Ser	Asn	Met	Val	Arg	Gly	Ile	Ile	Ile	Ala	Tyr	Tyr	Phe	Asp
1				5					10					15	

Tyr

&lt;210&gt; 82

&lt;211&gt; 17

&lt;212&gt; PRT

&lt;213&gt; Synthetic

&lt;220&gt;

&lt;221&gt; misc\_feature

<223> X is any amino acid different from the amino acid normally r  
eside

nt at that positio

&lt;400&gt; 82

Asp	Ser	Xaa	Asn	Met	Val	Arg	Gly	Ile	Ile	Ile	Ala	Tyr	Tyr	Phe	Asp
1				5					10					15	

Tyr

&lt;210&gt; 83

&lt;211&gt; 17

&lt;212&gt; PRT

&lt;213&gt; Synthetic



## A-633A PCT.txt

<220>  
 <221> misc\_feature  
 <223> X is any amino acid different from the amino acid normally r  
 eside  
 nt at that positio

<400> 83

Asp Ser Ser Xaa Met Val Arg Gly Ile Ile Ile Ala Tyr Tyr Phe Asp  
 1 5 10 15

Tyr

<210> 84  
 <211> 17  
 <212> PRT  
 <213> Synthetic

<220>  
 <221> misc\_feature  
 <223> X is any amino acid different from the amino acid normally r  
 eside  
 nt at that positio

<400> 84

Asp Ser Ser Asn Xaa Val Arg Gly Ile Ile Ile Ala Tyr Tyr Phe Asp  
 1 5 10 15

Tyr

<210> 85  
 <211> 17  
 <212> PRT  
 <213> Synthetic

<220>  
 <221> misc\_feature  
 <223> X is any amino acid different from the amino acid normally r  
 eside  
 nt at that positio

<400> 85

Asp Ser Ser Asn Met Xaa Arg Gly Ile Ile Ile Ala Tyr Tyr Phe Asp  
 1 5 10 15

Tyr

## A-633A PCT.txt

<210> 86  
 <211> 17  
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Tyr

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Tyr

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 eside

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nt at that positio

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Tyr

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nt at that positio

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Tyr

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nt at that positio

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Tyr

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/05973

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/13 C07K16/28 C07K16/46 C12N15/85 C12N5/10  
A61K39/395 A61P19/10 A61P35/00 A61K38/22  
//(A61K39/395, 38:22)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, CHEM ABS Data, WPI Data, PAJ, EPO-Internal, STRAND

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 46751 A (AMGEN, INC.) 22 October 1998 (1998-10-22) cited in the application page 23, line 28 -page 25, line 4 example 11 claims 25,26,34,36  -/-	1-7,21, 27-33

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

18 June 2001

Date of mailing of the international search report

06/08/2001

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/05973

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	N. TAKAHASHI ET AL.: "A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANSE/RANKL, regulates osteoclast differentiation and function." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 256, no. 3, 24 March 1999 (1999-03-24), pages 449-455, XP000877384 Duluth, MN, USA page 453, right-hand column, line 4 - line 6	1-7, 21, 27-33
X	K. TSUKII ET AL.: "Osteoclast differentiation factor mediates an essential signal for bone resorption induced by 1 alpha,25-dihydroxyvitamin D3, prostaglandin E2, or parathyroid hormone in the microenvironment of bone." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 246, no. 2, 19 May 1998 (1998-05-19), pages 337-341, XP002169940 Duluth, MN, USA the whole document	1, 2, 5-7, 27-33
X	WO 98 28424 A (IMMUNEX CORPORATION) 2 July 1998 (1998-07-02) example 10	1-7, 21, 27, 28
A	L. HOFBAUER ET AL.: "Osteoprotegerin and its cognate ligand: a new paradigm of osteoclastogenesis." EUROPEAN JOURNAL OF ENDOCRINOLOGY, vol. 139, no. 2, August 1998 (1998-08), pages 152-154, XP000876579 the whole document	1-53
P, X	WO 00 15807 A (M & E BIOTECH AS) 23 March 2000 (2000-03-23) page 53, line 30 - page 54, line 8	1-8, 21, 27-33
P, A	Y-Y. KONG ET AL.: "Osteoprotegerin ligand: a regulator of immune responses and bone physiology." IMMUNOLOGY TODAY, vol. 21, no. 10, October 2000 (2000-10), pages 495-502, XP004217459 Amsterdam, The Netherlands the whole document	1-53

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Information on patent family members

ional Application No

PCT/US 01/05973

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			EP 0946725 A	06-10-1999
			ES 2144386 T	16-06-2000
			WO 9828426 A	02-07-1998
			US 6017729 A	25-01-2000
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